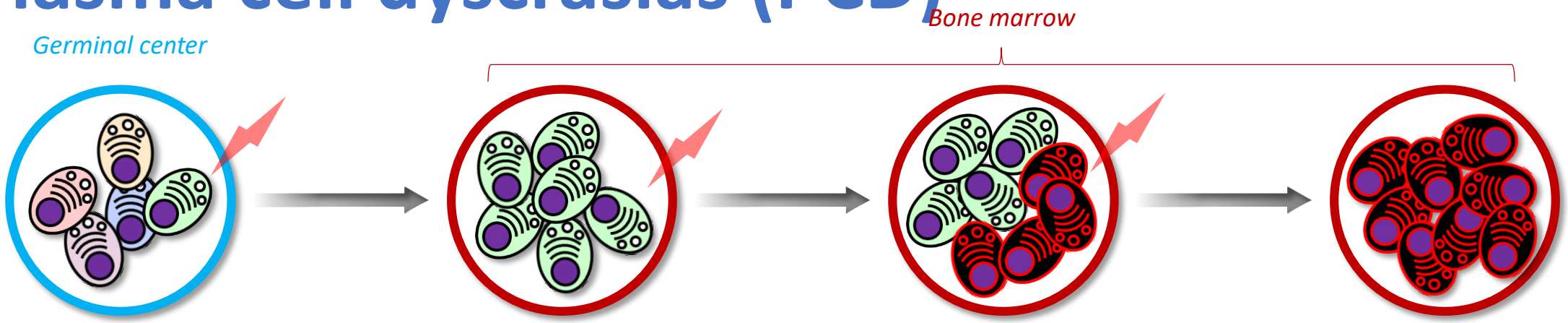
VII Curso de Actualización en Oncopatología y III Curso-Taller de Citometría de Flujo
Facultad de Medicina de la Universidad de Concepción
02 y 03 de mayo del 2019, Concepción, Chile

Enfermedad mínima residual en Mieloma Múltiple

Evangelina Agriello
Bahía Blanca, Argentina

Laboratorio de Especialidades Bioquímicas

Plasma cell dyscrasias (PCD)



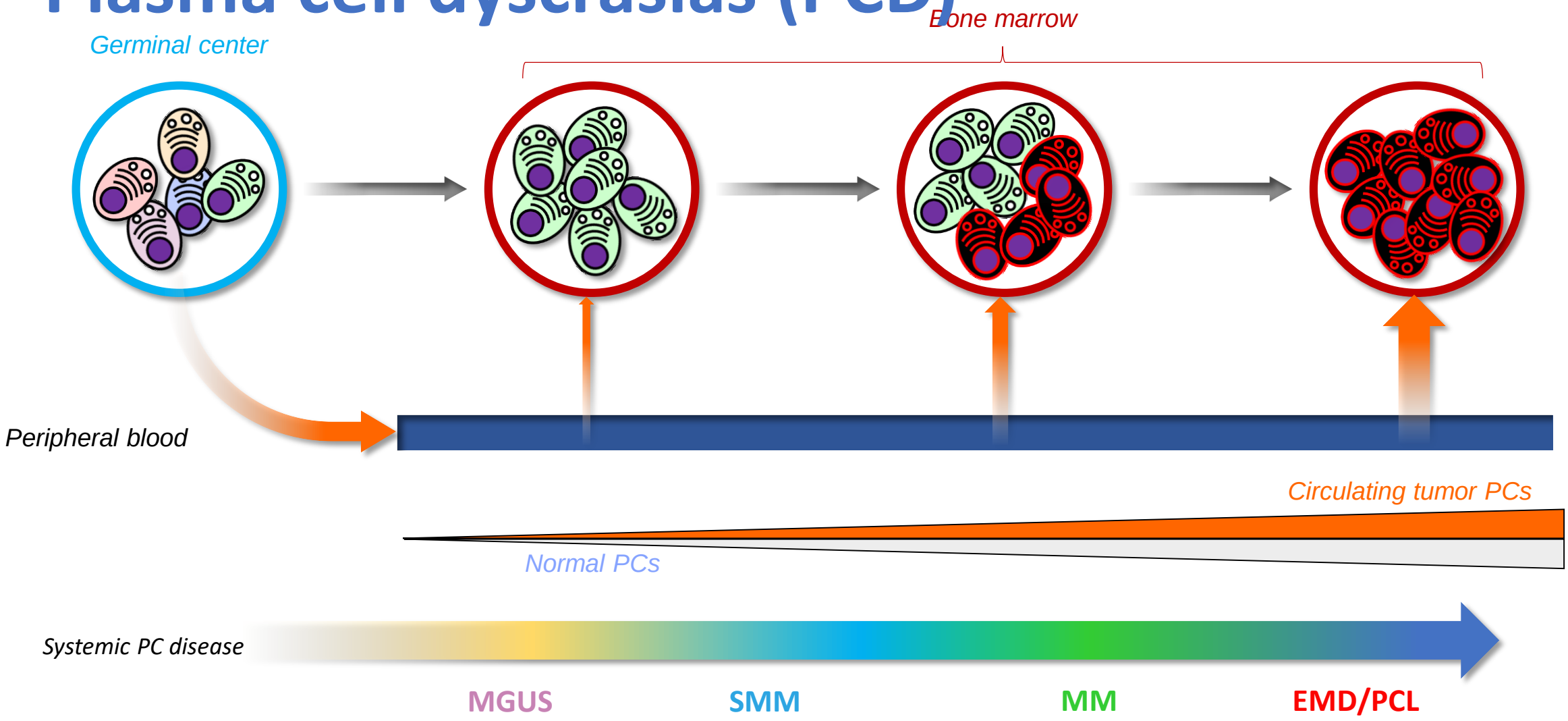
CONSECUTIVE GENETIC LESIONS

Systemic PC disease

MGUS SMM MM EMD/PCL

Sanoja-Flores L, et. al.; EuroFlow consortium, Blood Cancer J. 2018 Nov 19;8(12):117

Plasma cell dyscrasias (PCD)

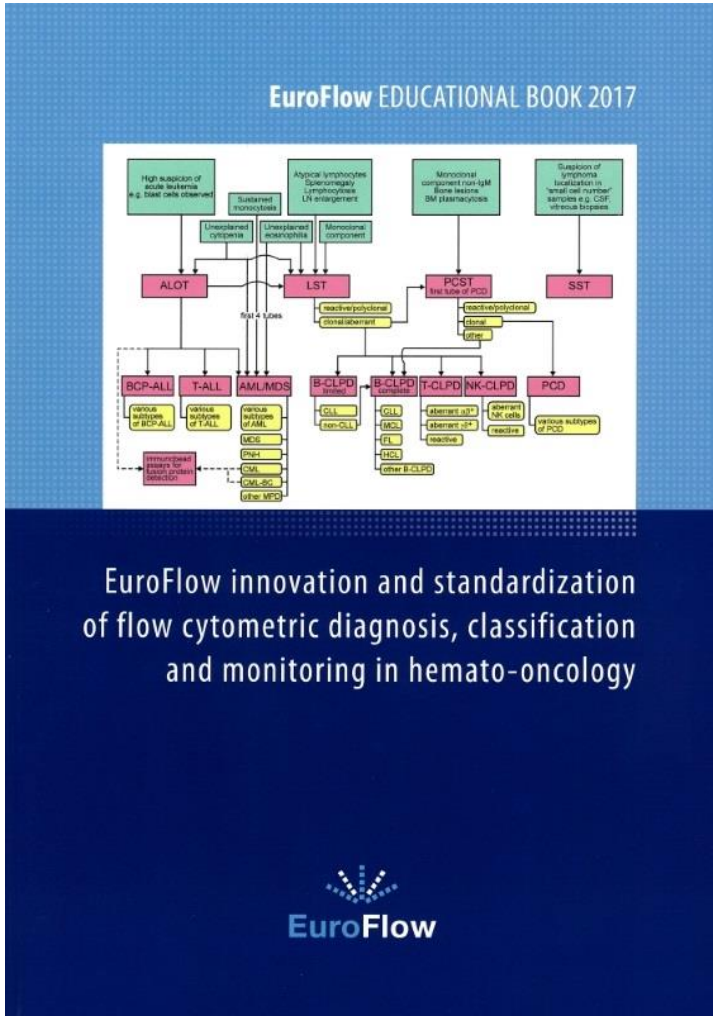


Sanoja-Flores L, et. al.; EuroFlow consortium, Blood Cancer J. 2018 Nov 19;8(12):117

Laboratory SOPs

Validated by an independent consortium of clinical flow cytometry experts – EuroFlow™

- Sample collection and transport
- Instrument set up, calibration and compensation
- Sample processing and staining

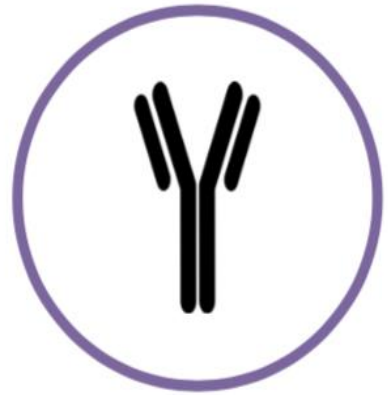


Available at www.euroflow.org

EuroFlow
a division of ESLHO

Pre-analytical Phase

- Specimen handling
- Anticoagulant
- Collection timing
- Preservation conditions



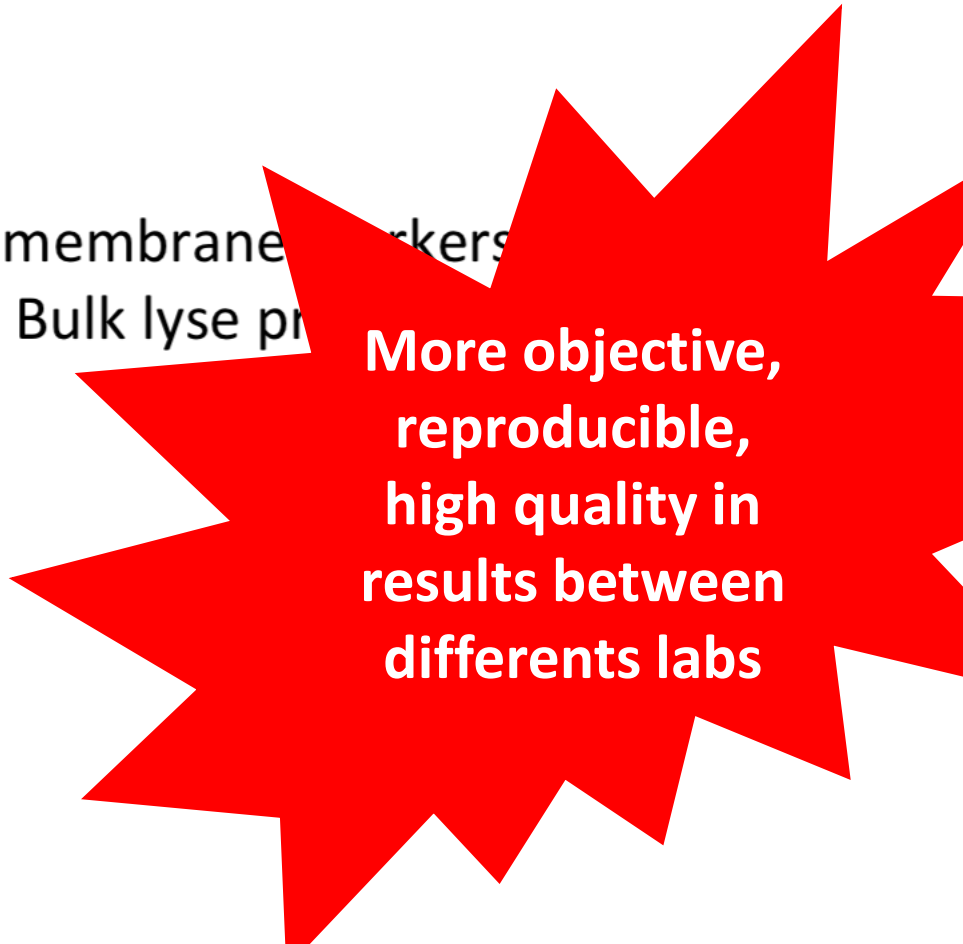
Analytical Phase

- Fluorochrome Selection
- Antibody combinations
- Sample preparation
- Instrument set-up
- Raw data generation

Post-analytical Phase

- Data integration
- Reporting

- Panel design
  - Fluorochromes
  - Antibodies
- Sample preparation and staining
  - Staining of surface markers only
  - Staining of surface markers including Igs
  - Combined staining of Intracellular and Surface membrane [illegible]rkers
  - Low cellularity samples/rare populations – The Bulk lyse pr[illegible]
- SOPs for instrument settings
  - FSC, SSC
  - Fluorescence channels
- SOPs for compensation settings



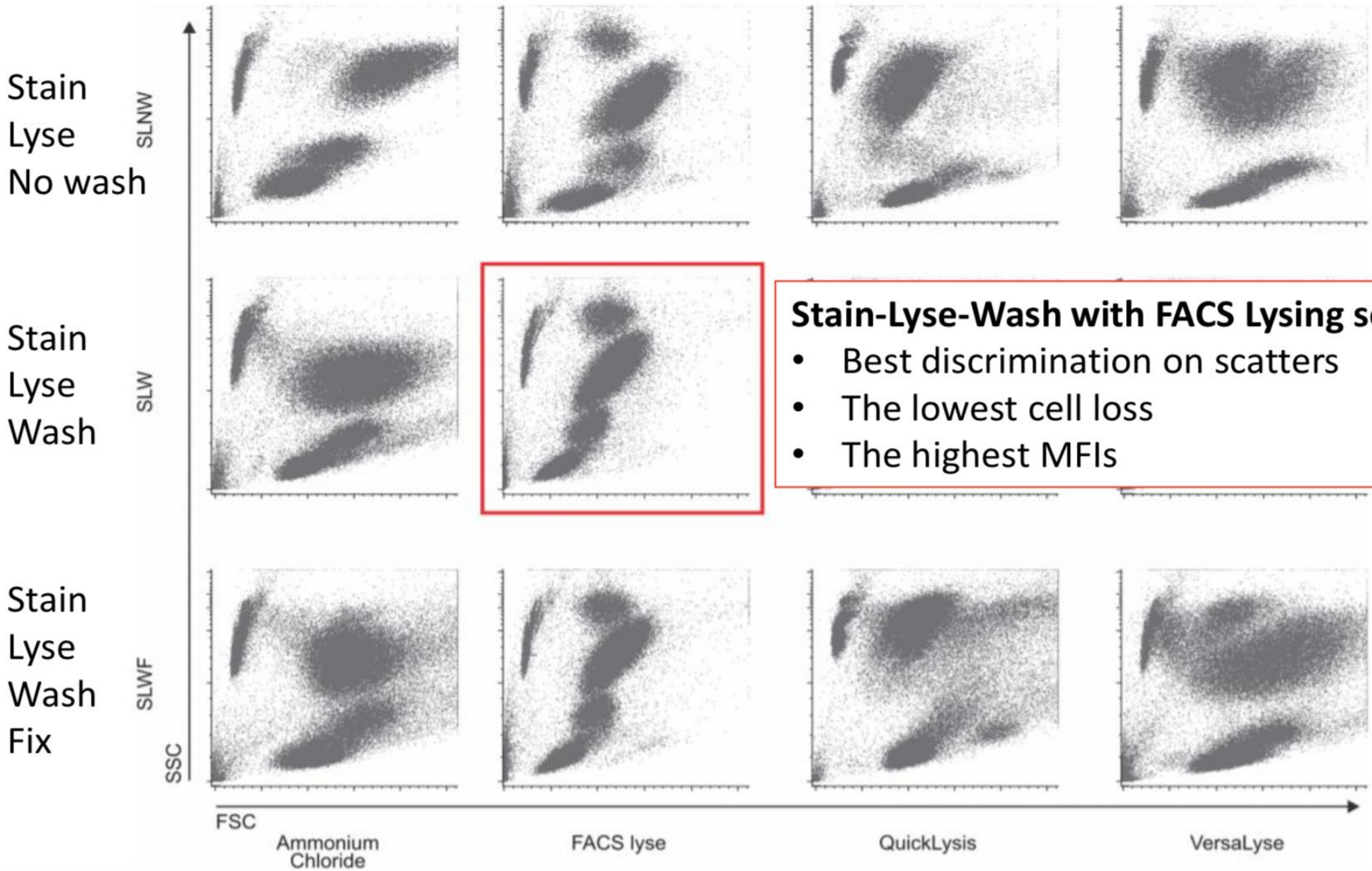
- Preselected - previous experience:

FITC, PE, PerCP, PerCP-Cy5.5, PECy7
APC

- Extensive testing - based on
  - ✓MFI
  - ✓Stain index
  - ✓Spillover
  - ✓Stability
  - ✓Availability

APC-Cy7, AF700, **APC-H7**,
PacB, HV450, **PacO**, AmCyan, HV500

EuroFlow SOP for sample preparation



Stain-Lyse-Wash with FACS Lysing solution

- Best discrimination on scatters
- The lowest cell loss
- The highest MFIs

Add antibodies

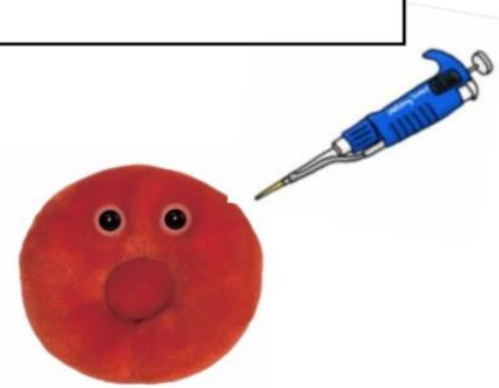
- Multiple tubes? First Abs

Wash buffer – final volume 50 µL

- **NaN_3 – Sodium azide**
  - Inhibits potential redistribution of surface antigens
- **BSA – Bovine serum albumin**
  - Prevents non-specific binding

Add 50 µL of sample

- Final volume 100 µL
- **Incubate 30 min**



FACS Lysing solution

- 1:10 distilled water
- **Incubate 10 min**

Spin → wash → spin

- 540g, 5 min
- **Resuspend** 2mL (no NaN_3)

Resuspend → Acquire

- Within an hour! 4°C, dark

EuroFlow SOPs: combined intracellular and surface membrane staining

- **ALOT, MM MRD tube 2, PCST**

Membrane staining

Add antibodies	• Multiple tubes? First Abs

Wash buffer – final volume 50 µL	• **NaN_3 – Sodium azide**
	  • Inhibits potential redistribution of surface antigens
	• **BSA – Bovine serum albumin**
	  • Prevents non-specific binding

Add 50 µL of sample	• Final volume 100 µL • **Incubate 30 min**

Fix

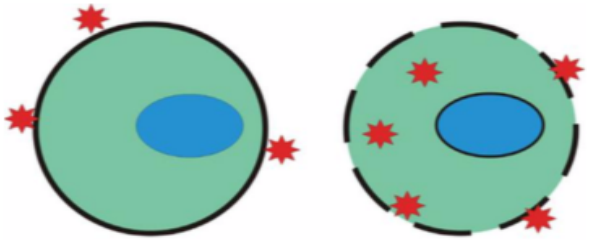
- Solution A, **MIX**
- 15 min, RT, dark

Wash → spin

- 540g, 5 min
- Resuspend

Permeabilize

- Solution B, **MIX**
- 15 min, RT, dark



Stain

- Intracellular
- 15 min, RT, dark

Spin -> **wash**-> spin	• 540g, 5 min • **Resuspend** 2mL wash buffer
Resuspend -> **Acquire**	• Without NaN3 • Within an hour! 4°C, dark

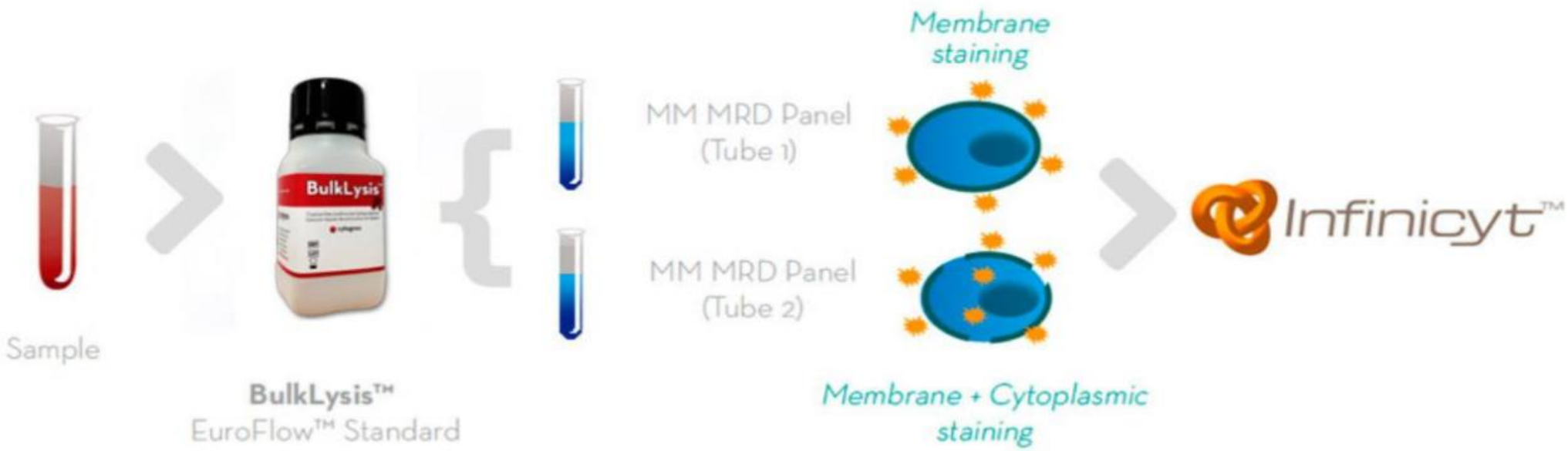
cytognos
Flow Cytometry Solutions

Reagents Infinicyt™ Support

EuroFlow SOPs – BulkLysis protocol

Why? To increase the sensitivity of MRD detection or when higher nucleated cell concentration is needed

- ✓MRD in Multiple Myeloma (MM)
- ✓MRD in B-cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL)
- ✓Primary Immunodeficiencies Orientation Tube (PIDOT)



EuroFlow SOPs – BulkLysis protocol

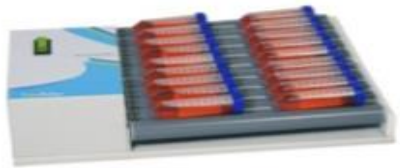
Sample in 50 mL tube

- Max 2mL in one tube
- MRD: min 10 x 10^6 nucleated cells per tube

Fill the tube up to 50 mL with Bulk lyse

- **diluted** to 1X in dH2O at **RT**

15 min roller

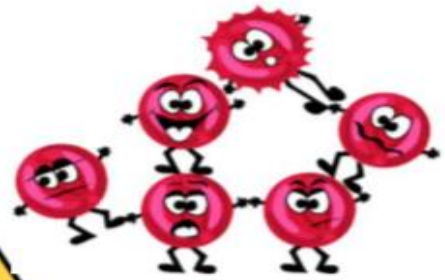


800g, 10min → discard supernatant

- ~ 300 µL remains

Resuspend → Wash

- Add 2 mL wash buffer → **resuspend** → fill up to 50 mL wash buffer



800g, 5min → discard supernatant

- ~ 300 µL remains

EuroFlow SOPs – BulkLysis protocol

Resuspend → Transfer to FACS tube

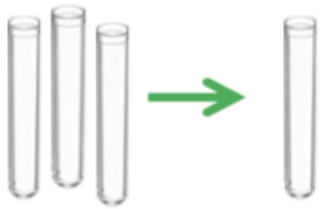
- Add 2 mL wash buffer → **resuspend**

Recover the rest of the cells

- Wash the Falcon tube with 2 mL wash buffer → transfer to the FACS tube

540g, 5 min → discard supernatant

- If more tubes were used → combine
- Final volume ≥ 300 µL



Adjust concentration!

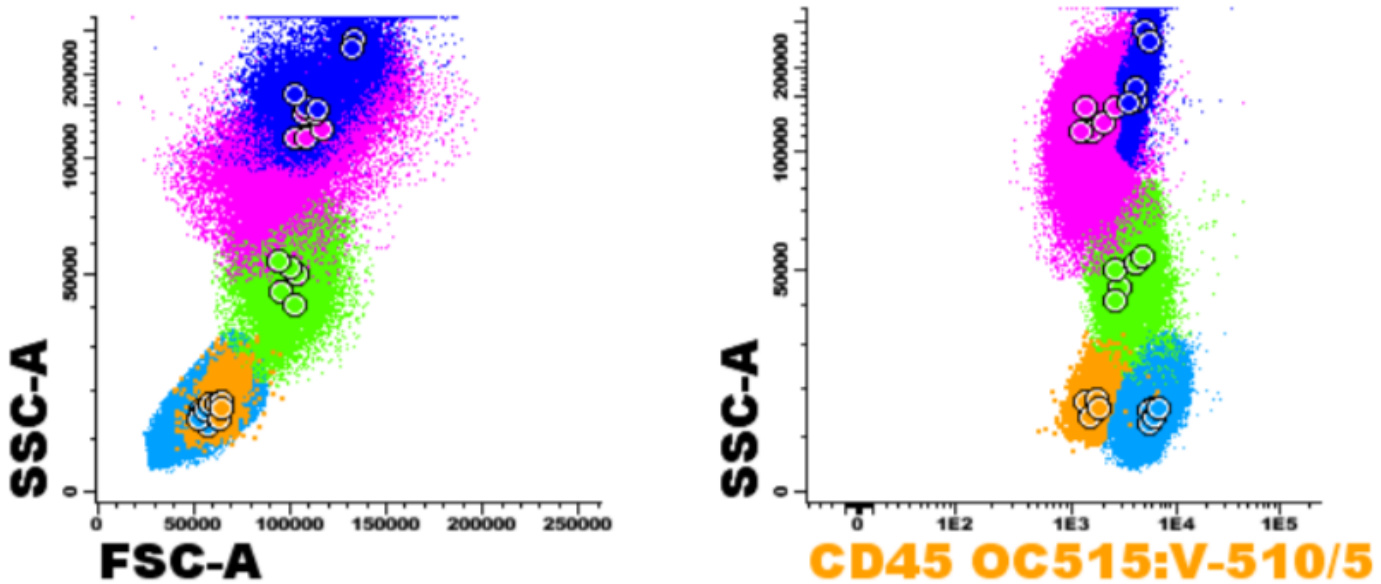
- **1 x 10^5 cells/µL**

FOLLOW APPROPRIATE **STAINING PROTOCOL:**

- PIDOT, BCP-ALL MRD, MM MRD Tube 1: membrane staining
- MM MRD tube 2: intracellular staining

SOP for standardized instrument setttings – FSC + SSC

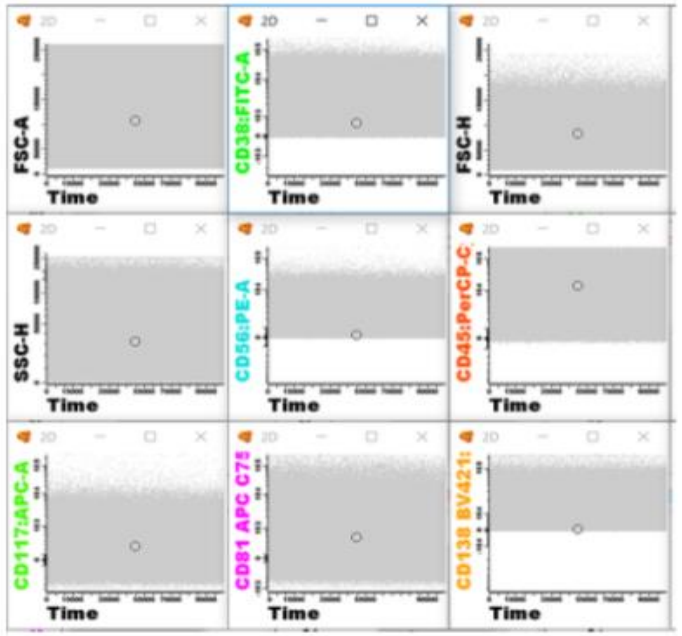
BD FACSCanto II:
FSC: 55,000 (range 50,000 – 60,000)
SSC: 13,000 (range 11,000 – 15,000)
Threshold in FSC: 10,000



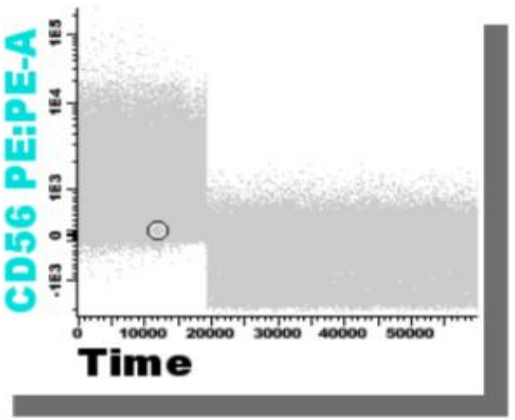
- Gating
- Comparison
- Follow up
- Database
- Merge

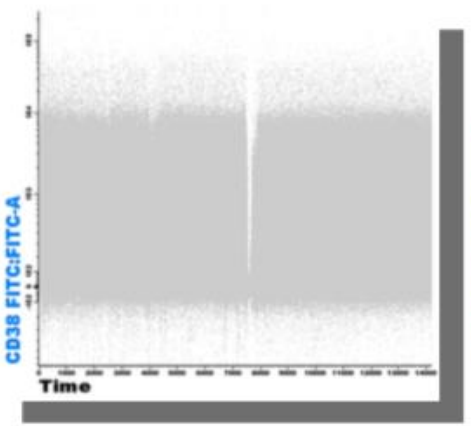
5x normal PB stained with LST, merged

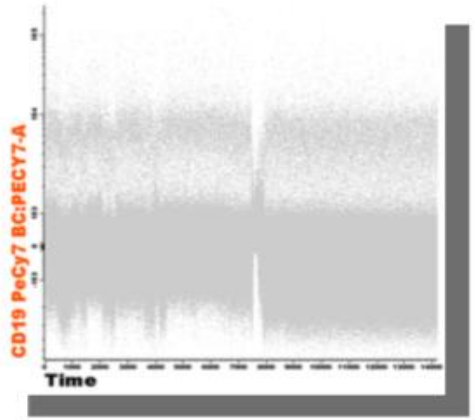
A. Record time parameter to detect technical issues



System fluctuations (laser, sheath pressure)?
Cell aggregation/clumping?
Bubbles?







E. Anticoagulants affect the expression of certain markers

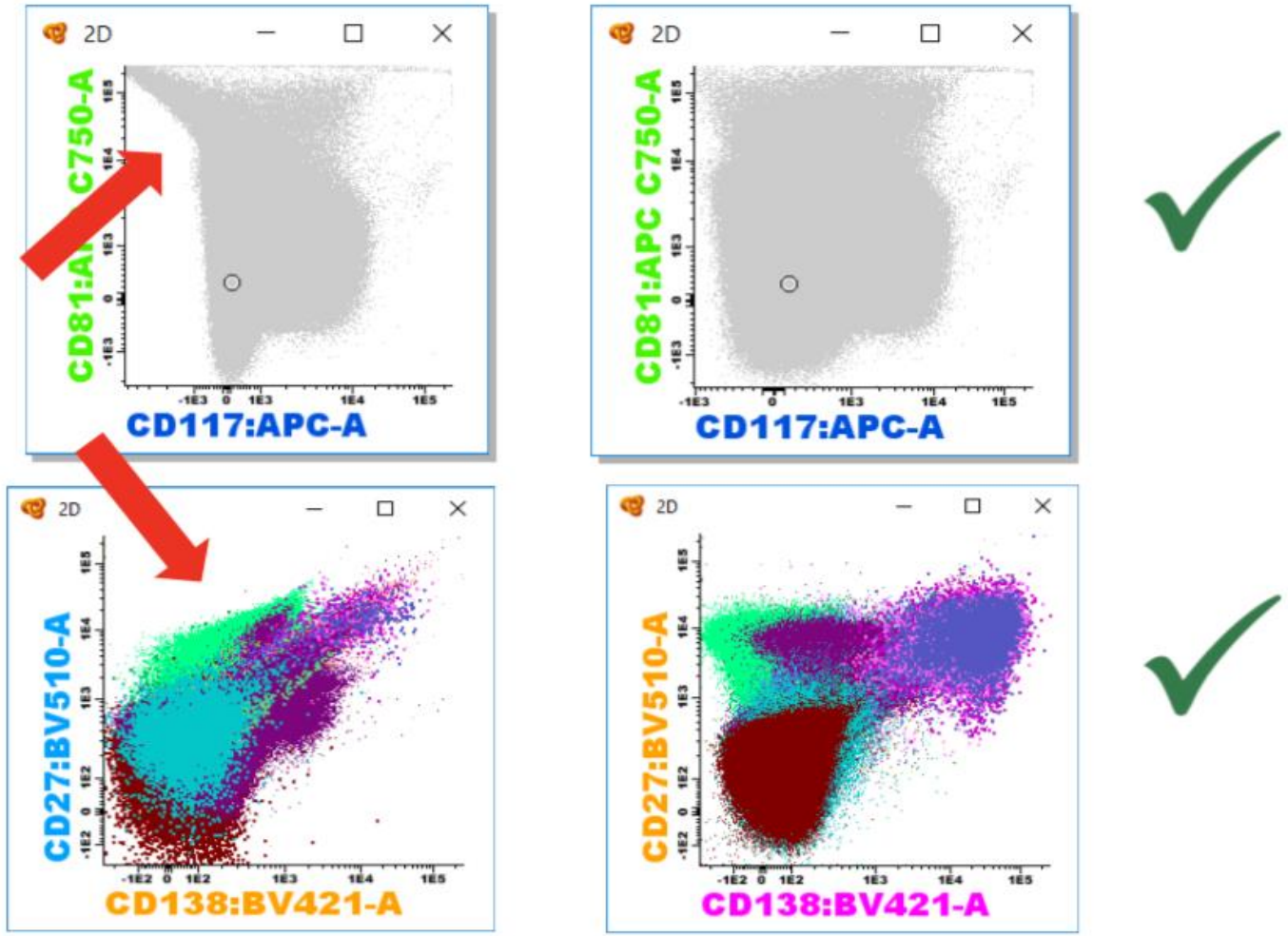
- EuroFlow method has been validated with samples collected with **EDTA** anticoagulant (purple top tube)

- The use of other anticoagulants, e.g. **heparin**, affects the expression of certain markers

Downregulation of CD138 expression in the membrane of plasma cells after heparin exposure

Flores-Montero J, de Tute R, Paiva B, Perez JJ, Böttcher S, Wind H, Sanoja L, Puig N, Lecrevisse Q, Vidriales MB, van Dongen JJM and Orfao A. ***Immunophenotype of Normal vs. Myeloma Plasma Cells: Toward Antibody Panel Specifications for MRD Detection in Multiple Myeloma****. Cytometry Part B 2016; 90B: 61–72.*

D. Compensation issues



Use single-stained controls (cells or beads) with **same fluorochrome** as used in the assay
Unstained control must have **same autofluorescence** as single-stained controls
Tandem dyes need **lot specific compensation**

Plasmatic Cells Screening Tube

- **Consensus 8-color panel:**

PacB	OC515	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
CD45	CD138	CD38	CD56	$\beta_2$microglobulin	CD19	CyIgKappa	CyIgLambda
CD45*	CD138*	CD38	CD28	CD27	CD19	CD117	CD81

V450	V500c	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
CD138	CD45	CD38	CD56	$\beta_2$microglobulin	CD19	CyIgKappa	CyIgLambda
CD138	CD45	CD38	CD28	CD27	CD19	CD117	CD81

- Identification, discrimination and enumeration of normal and aberrant plasma cells
- Initial evaluation and screening of PCD in BM simples
- B_2-microglobulin allows for prognostic stratification

Leukemia (2017) **31,** 2094–2103

www.nature.com/leu

OPEN

ORIGINAL ARTICLE

Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma

J Flores-Montero[1,19], L Sanoja-Flores[1,19], B Paiva[2,19], N Puig[3], O García-Sánchez[3], S Böttcher[4], VHJ van der Velden[5], J-J Pérez-Morán[3], M-B Vidriales[3], R García-Sanz[3], C Jimenez[3], M González[3], J Martínez-López[6], A Corral-Mateos[1], G-E Grigore[7], R Fluxá[7], R Pontes[8], J Caetano[9], L Sedek[10], M-C del Cañizo[3], J Bladé[11], J-J Lahuerta[6], C Aguilar[12], A Bárez[13], A García-Mateo[14], J Labrador[15], P Leoz[1], C Aguilera-Sanz[16], J San-Miguel[2,20], M-V Mateos[3,20], B Durie[17,21], JJM van Dongen[5,18,21] and A Orfao[1,21]

Reagent kit for MM MRD/CTPC

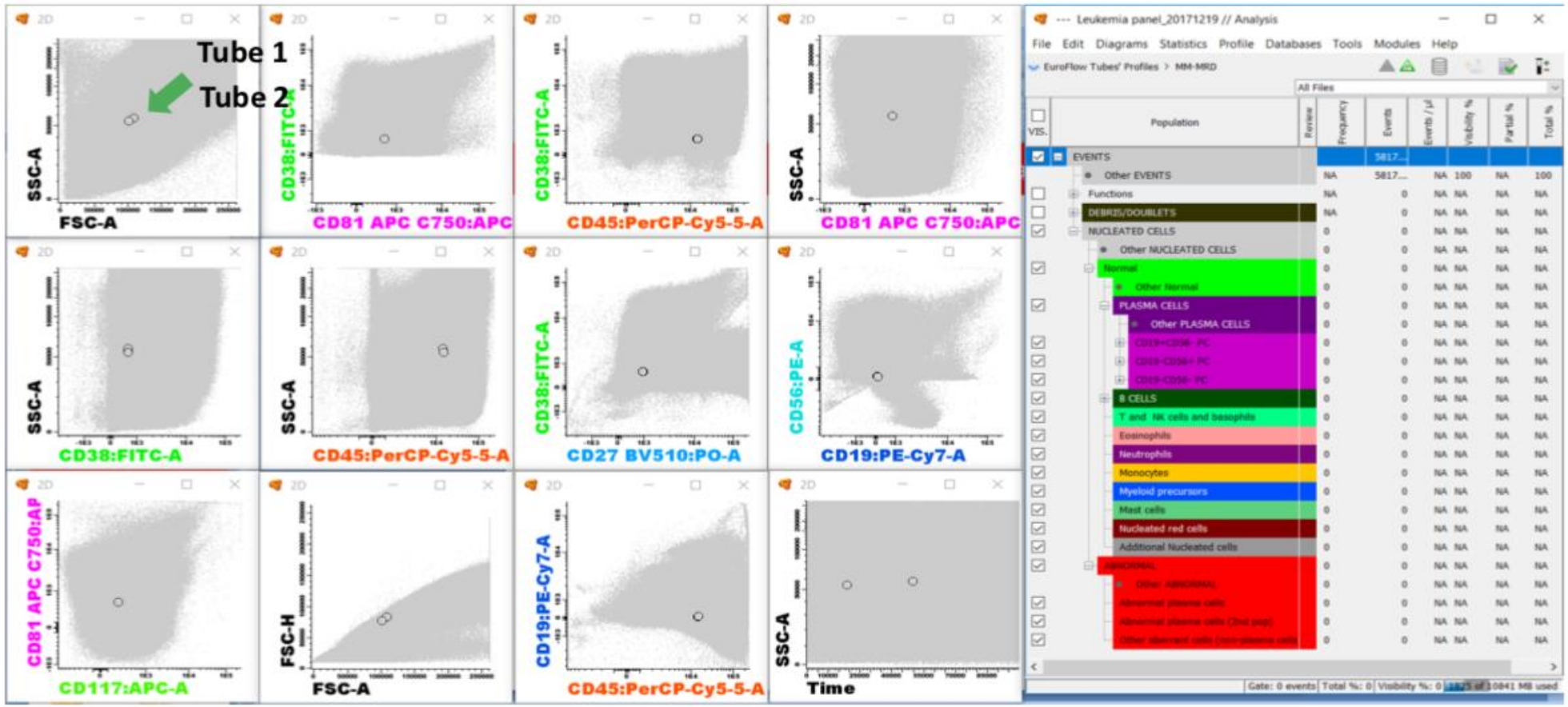
- **Consensus 8-color 2-tube panel:**

MM MRD	BV421	BV510	FITC	PE	PerCP-Cyanine 5.5	PE-Cyanine7	APC	APC-C750
Tube 1	CD138*	CD27*	CD38-multiepitope	CD56	CD45	CD19	CD117	CD81
Tube 2	CD138*	CD27*	CD38-multiepitope	CD56	CD45	CD19	CyIgKappa	CyIgLambda

- High sensitivity of close to 10^{-6} as recommended by the IMWG
- CD38 multiepitope to overcome antigen loss post-drug treatment
- Replicate consistency by the use of two tubes
- Pre-mix lyophilized format to avoid pipetting errors and to reduce time and costs
- Ancillary reagents included: BulkLysis™, FIX&PERM® , specific compensation vials
- Minimally-invasive test to evaluate tumor load in peripheral blood

**CD138-BV421 and CD27-BV510 to be added as drop-ins*

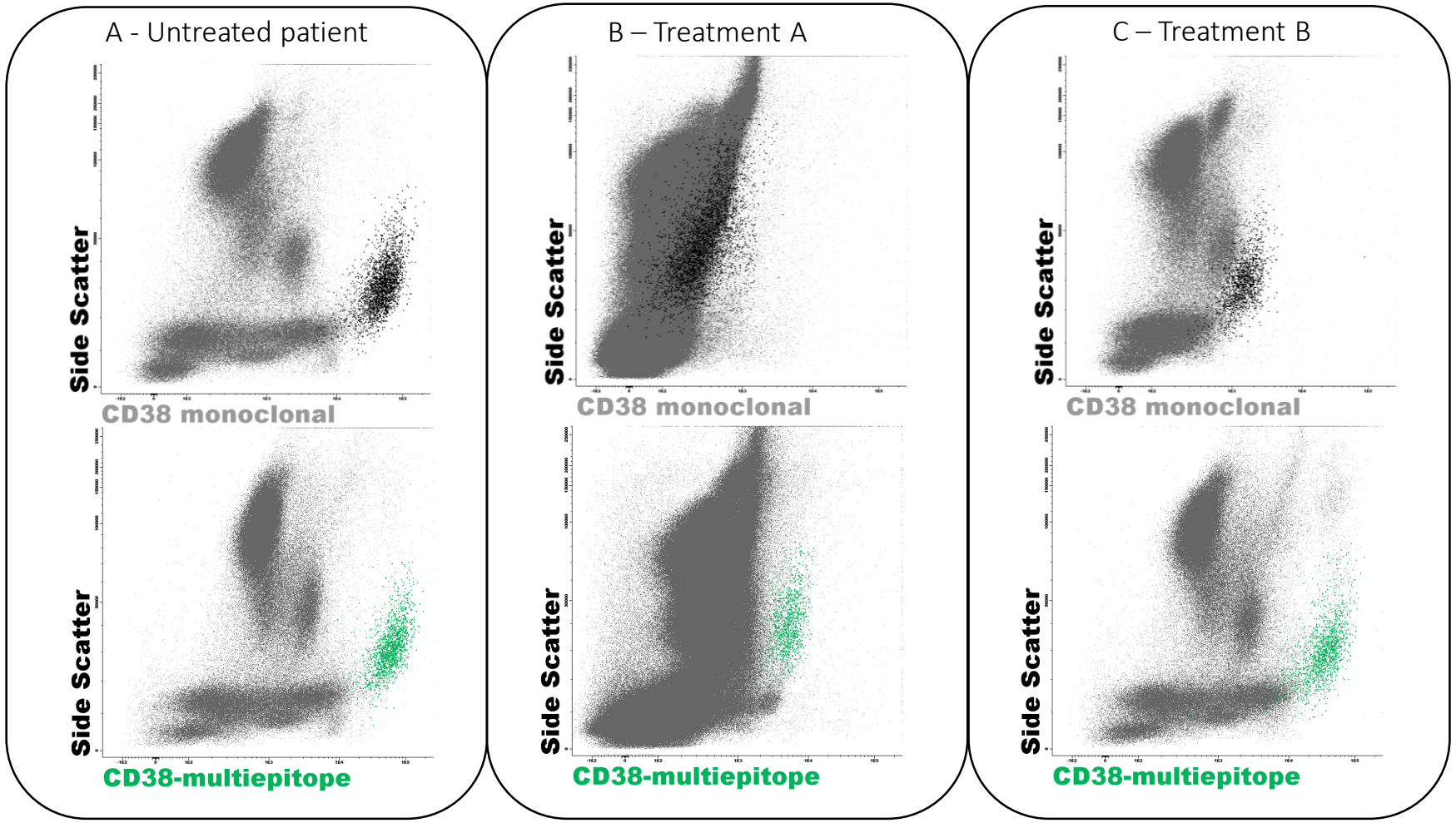
H. MM MRD: different processing Tube 1 vs tube 2

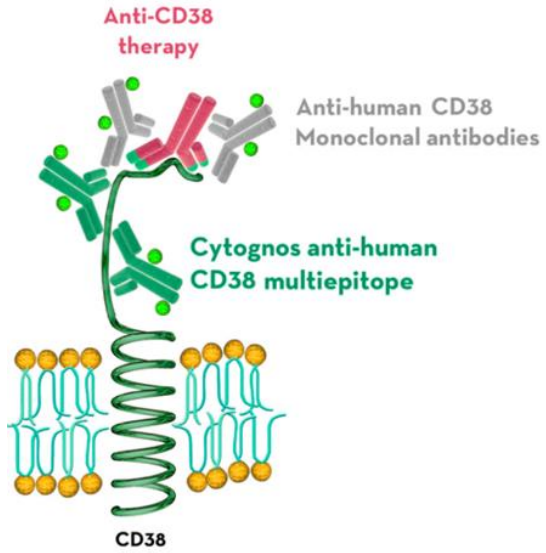


Median values from both tubes should look a bit different

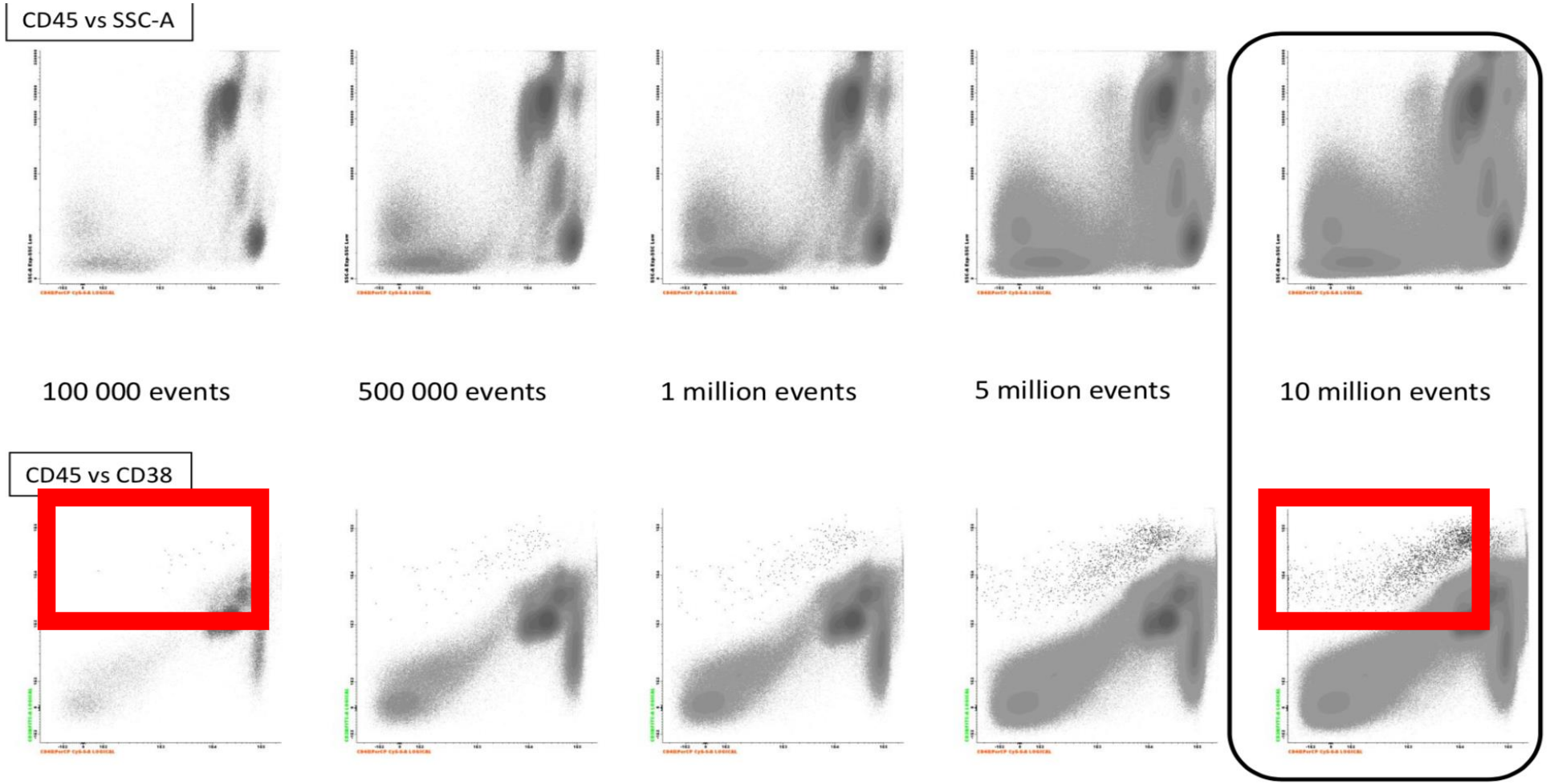
Treatment considerations: CD38 Multiepitope

Targets CD38 molecule on plasma cells, even in patients under anti-CD38 therapy.





Número de células colectadas es CRITICO para la sensibilidad del ensayo



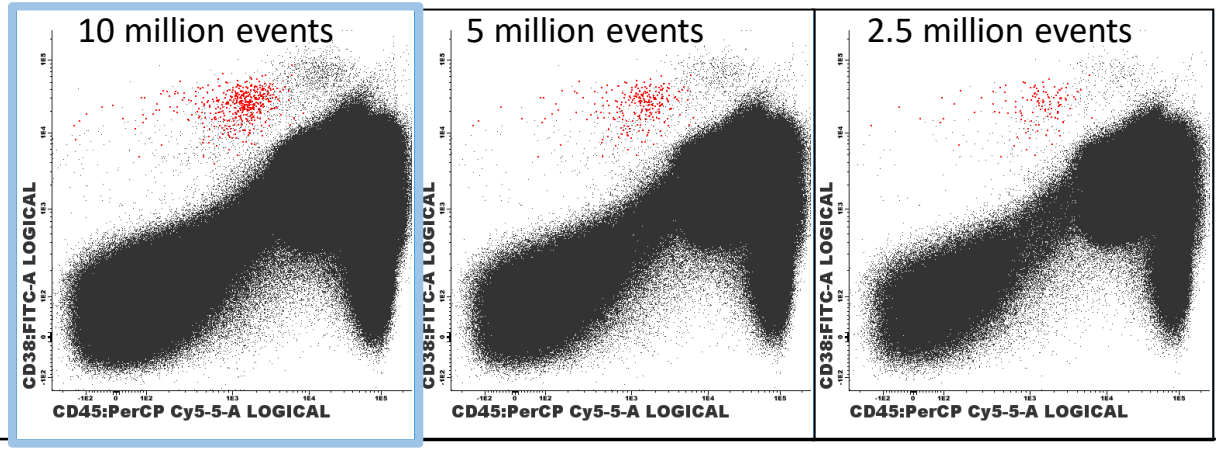
Sensitivity

IMWG recomendation: Sensitivity 10^{-5}

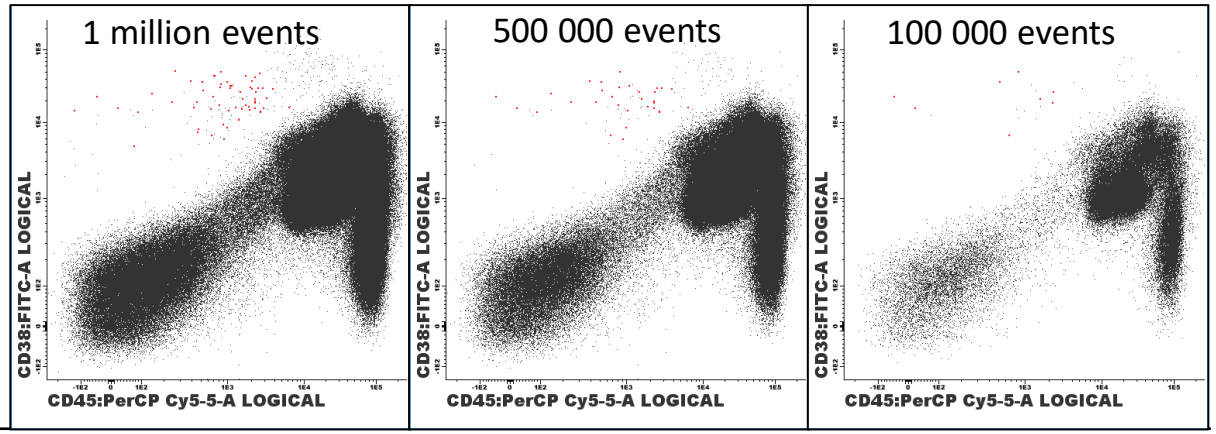
$$\%\ LOD = 100 \times \frac{20}{Nucleated\ cells}$$

$$\%\ LOQ = 100 \times \frac{50}{Nucleated\ \ cells}$$

$$\%\ cPC = 100 \times \frac{cPC}{Nucleated\ cells}$$



	10 million events	5 million events	2.5 million events
% LOD	0.0002	0.0004	0.0008
% LOQ	0.0005	0.0010	0.0021
% cPC	**0.0031**	**0.0016**	**0.0008**



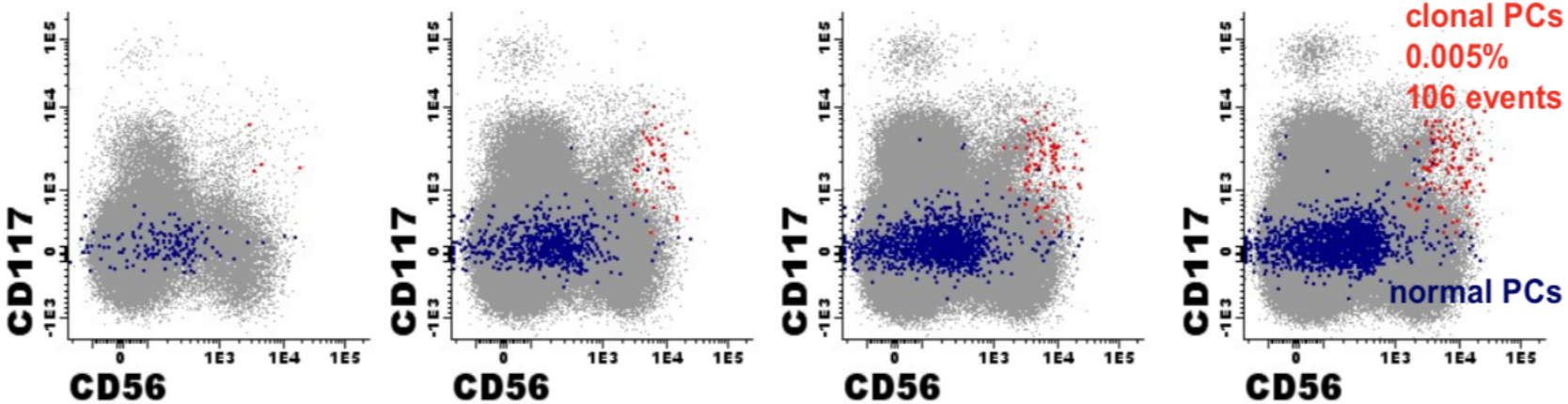
	1 million events	500 000 events	100 000 events
% LOD	0.0021	0.0041	0.0207
% LOQ	0.0052	0.0104	0.0518
% cPC	**0.0003**	**0.0002**	**0.0000**

International Myeloma Working Group (IMWG).

2016: MRD monitoring using 2nd generation flow affords higher sensitivity

1st generation flow
2X10^5 cells analyzed

2nd generation flow
2X10^6 cells analyzed

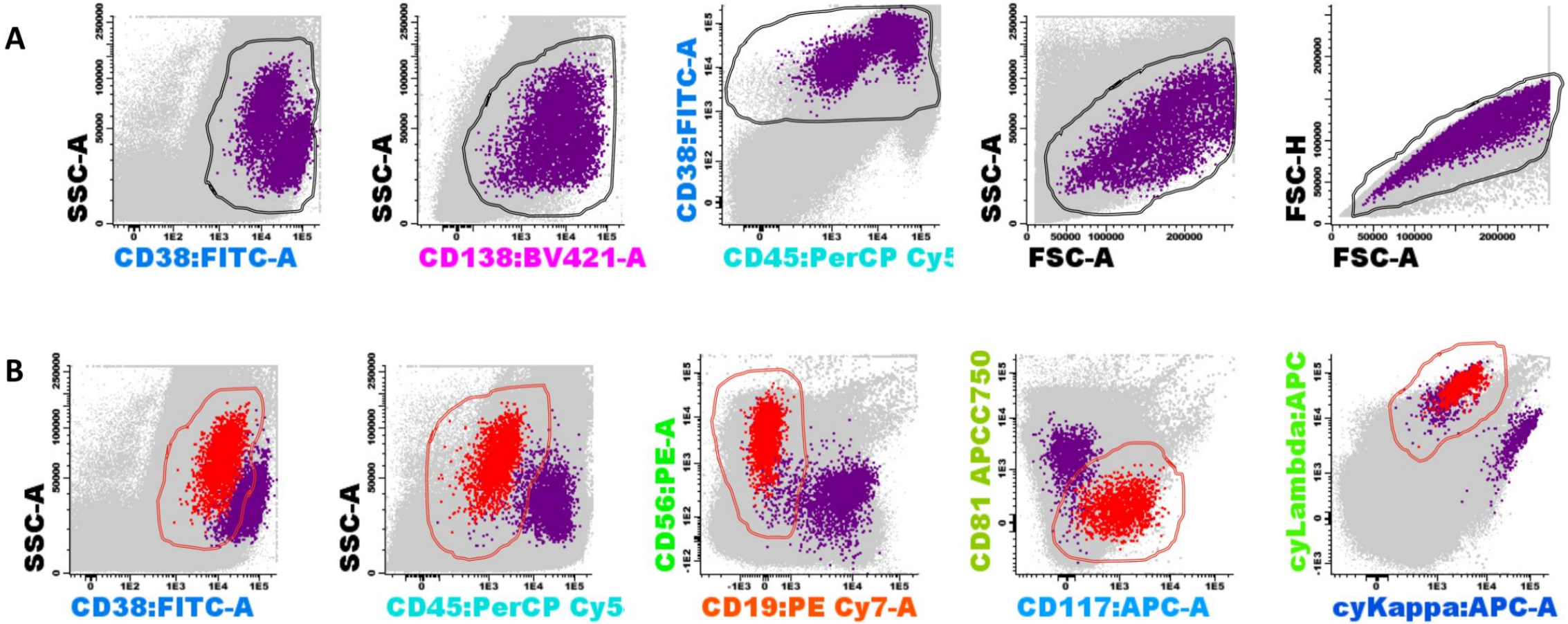


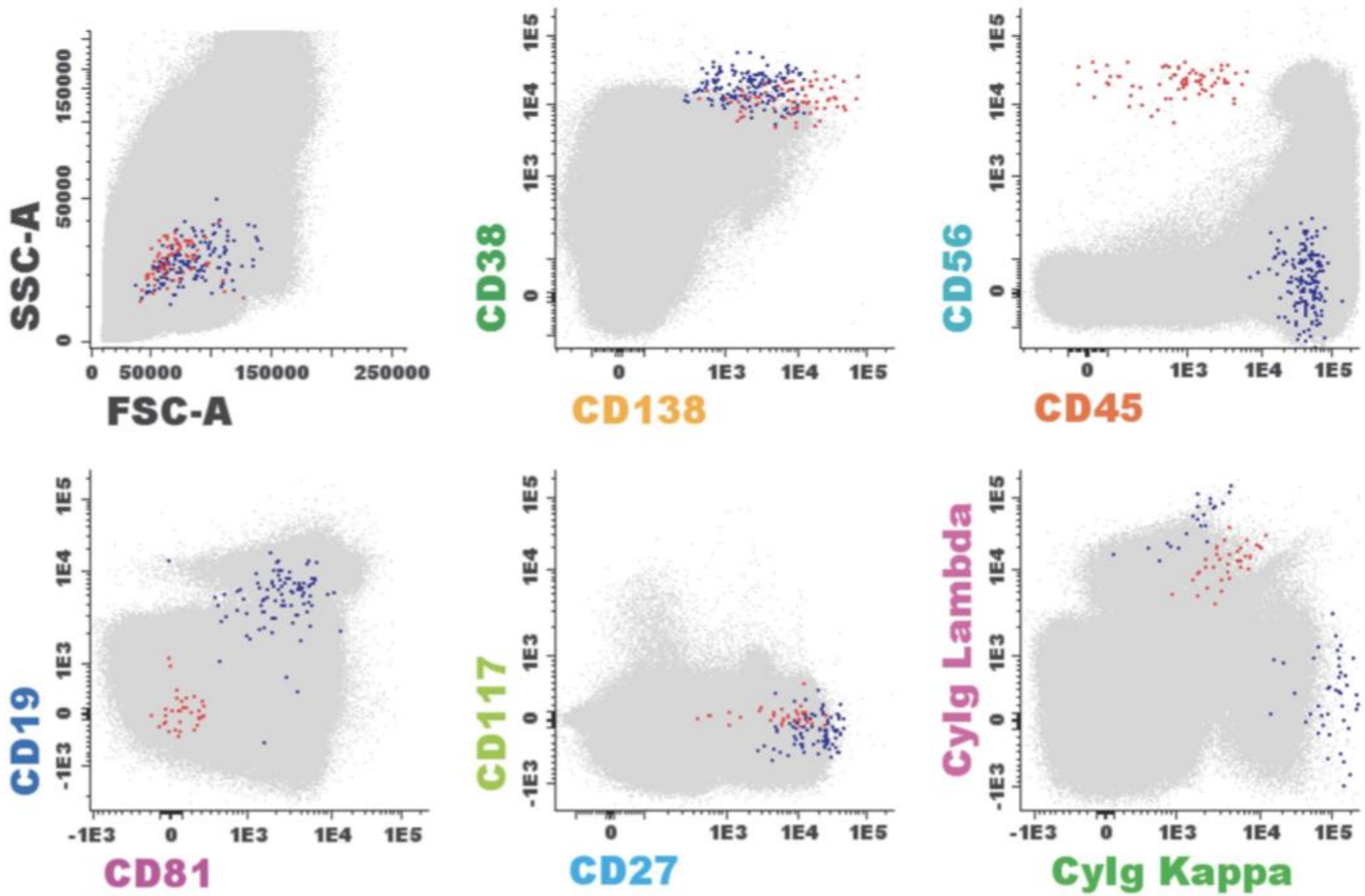
1st generation flow	2nd generation flow -	2nd generation flow +
-		15/50 (30%)
+	0/50 (0%)	35/50 (70%)

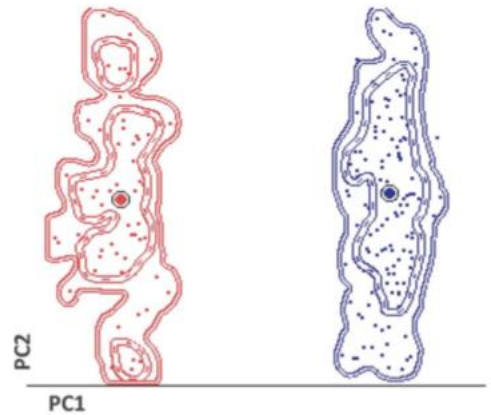
GEM2010MAS65

Paiva B et al. Blood 2016;127(25):3165-74

The gating strategy for the identification of a total plasma cell population and the discrimination of normal vs clonal plasma cells. An example of the sequential steps followed to gate the total plasma cell population are shown in Panel A. Panel B shows the discrimination of the clonal plasma cell population in this sample based on their abnormal phenotypic profile ($CD38^{lo}$, $CD45^{-}$, $CD19^{-}$, $CD56^{het+}$, $CD81^{-}$, $CD117^{+}$ and cyIg restriction $cyIg\lambda^{+}$ / $cyIg\kappa^{-}$). The total plasma cell population corresponds to 0.55% of BM cellularity while the clonal plasma cells represent 0.27%. Please note that the phenotypic profile of clonal plasma cells might be different in each patient sample.







Parameter	% of contribution
CD56	28%
CD19	19%
CD45	17%
CD81	16%
CD138	6%
CD27	4%

AUTOMATIC REPORT INFORMATION

Cellularity

Frequency of the populations in the bone marrow of study (% of events within the reference population). In bold, populations altered compared to the reference range.

Population	Frequency	Reference
Plasma cells	**0.029**	(0.048 - 0.97)
B cells	**0.38**	(1 - 5.2)
Mature B cells	**0.38**	(0.95 - 3.6)
T and NK and basophils	**25.7**	(8.8 - 17.4)
Eosinophils	1.1	(0.68 - 2.3)

Other features:

Absent populations: populations not present in the sample of study at the moment the report is opened. They can be in the unchecked events or not present at all. These populations should be found in a normal sample and are included in the database.

Although the analysis displays all absent populations, the report informs only if they are B-cell precursors, Mast cells, B cells, Mature B cells, T and NK and Basophils, Eosinophils, Neutrophils, Monocytes, Myeloid precursors, Nucleated red cells.

LYMPHOID NEOPLASIA

Detailed characterization of multiple myeloma circulating tumor cells shows unique phenotypic, cytogenetic, functional, and circadian distribution profile

Bruno Paiva,[1] Teresa Paino,[2] Jose-Maria Sayagues,[2,3] Mercedes Garayoa,[2] Laura San-Segundo,[2] Montserrat Martín,[2] Ines Mota,[3] María-Luz Sanchez,[2,3] Paloma Bárcena,[2,3] Irene Aires-Mejia,[2] Luis Corchete,[2] Cristina Jimenez,[2] Ramon Garcia-Sanz,[2] Norma C. Gutierrez,[2] Enrique M. Ocio,[2] Maria-Victoria Mateos,[2] Maria-Belen Vidriales,[2] Alberto Orfao,[2,3] and Jesús F. San Miguel[1]

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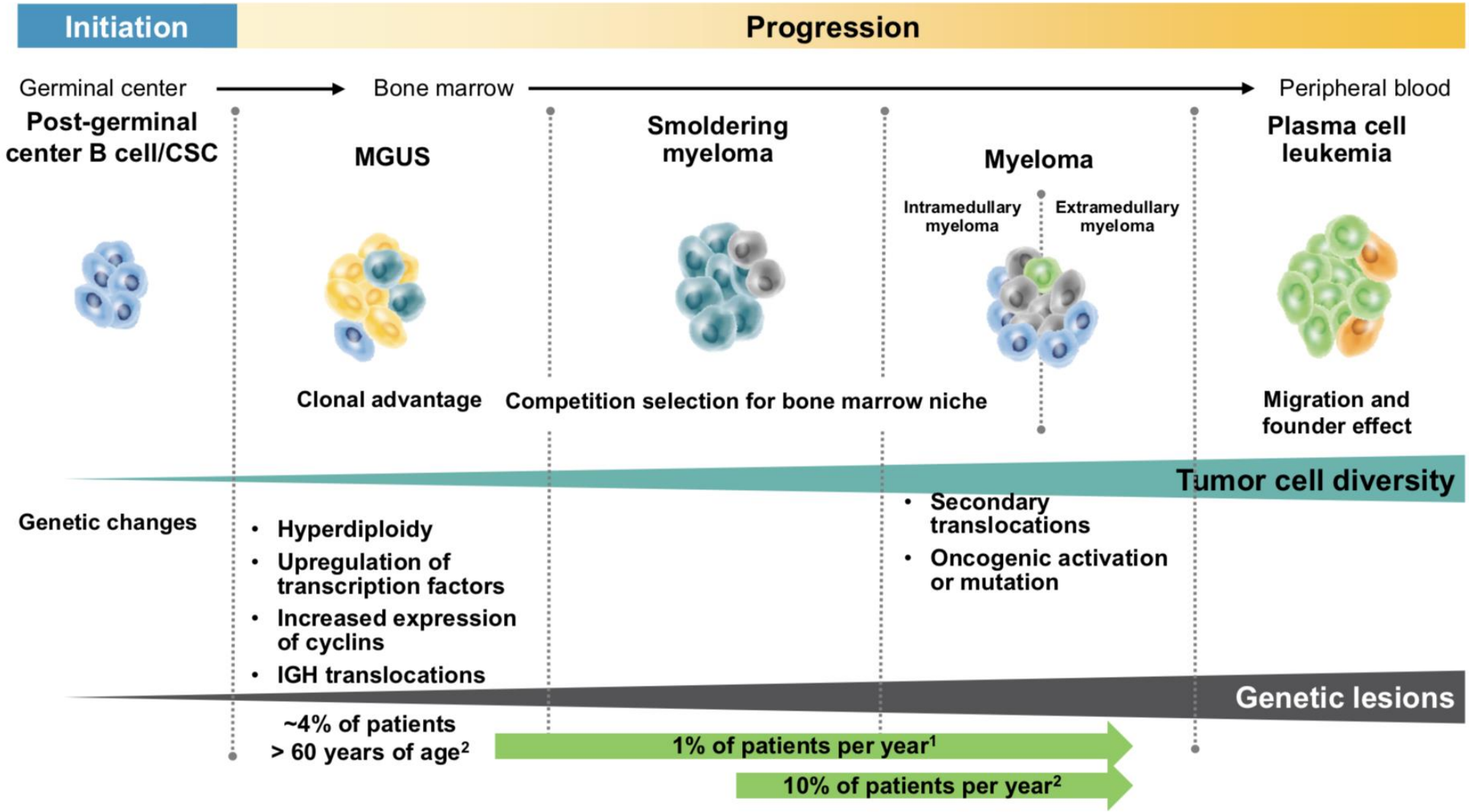
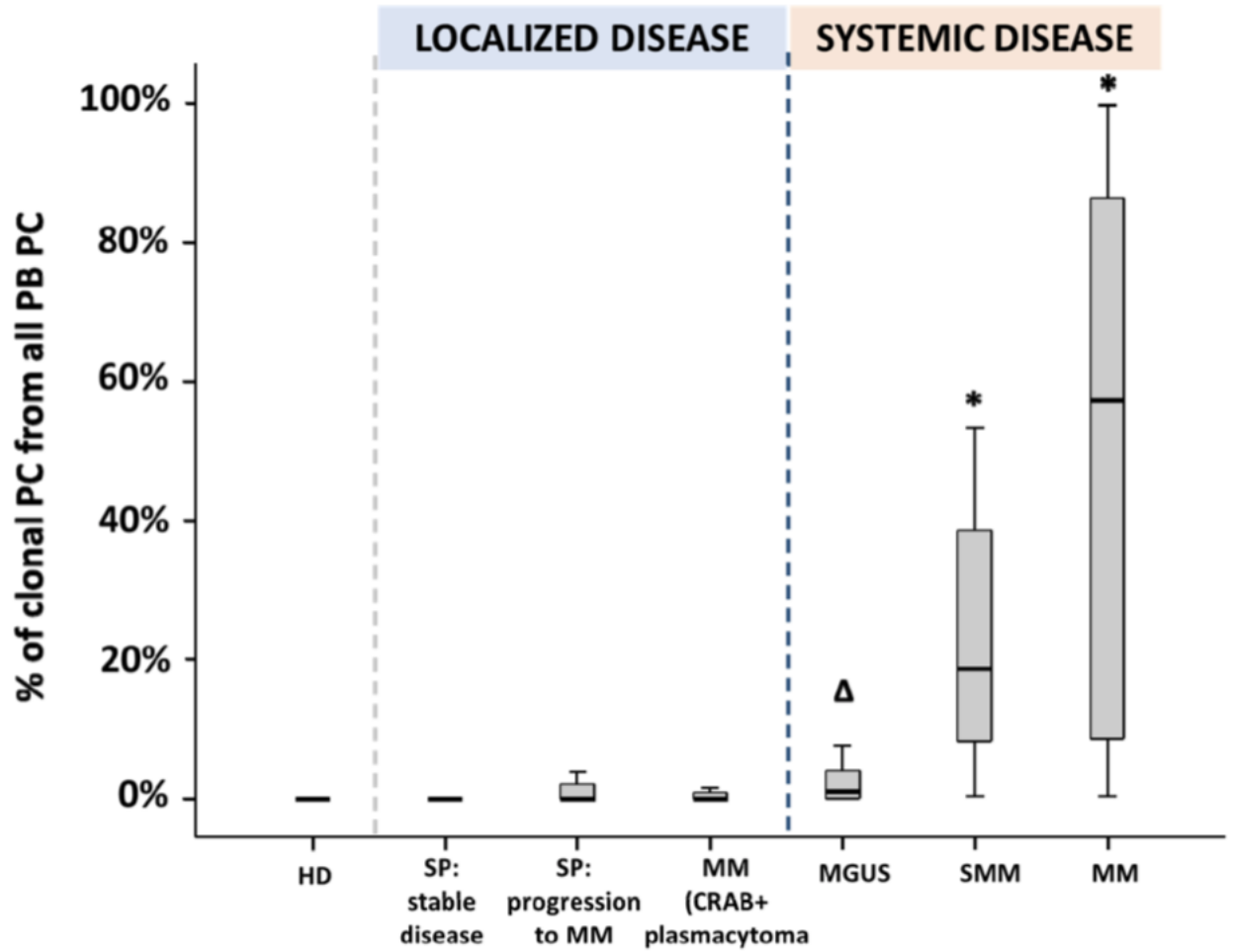


CSC, cancer stem cell; IGH, immunoglobulin heavy chain; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma.

1. Kyle RA, et al. N Engl J Med. 2002;346:564-569.
2. Kyle RA, et al. N Engl J Med. 2007;356:2582-2590.

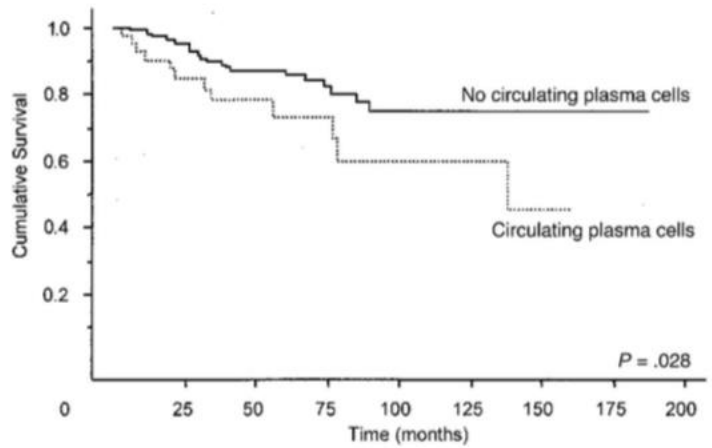
Circulating tumor cells (CTCs) progressively increase from MGUS to MM



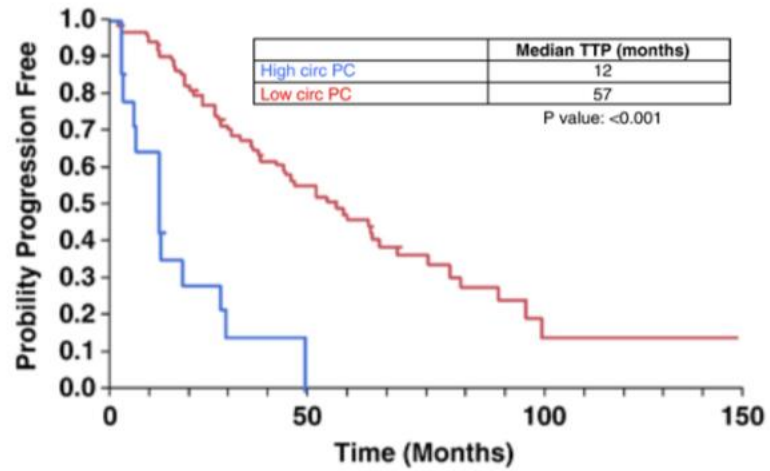
Sanoja-Flores L, et al. Blood 2015;126: 4180

Clinical significance of CTCs quantification in MGUS and smoldering MM

MGUS [1]

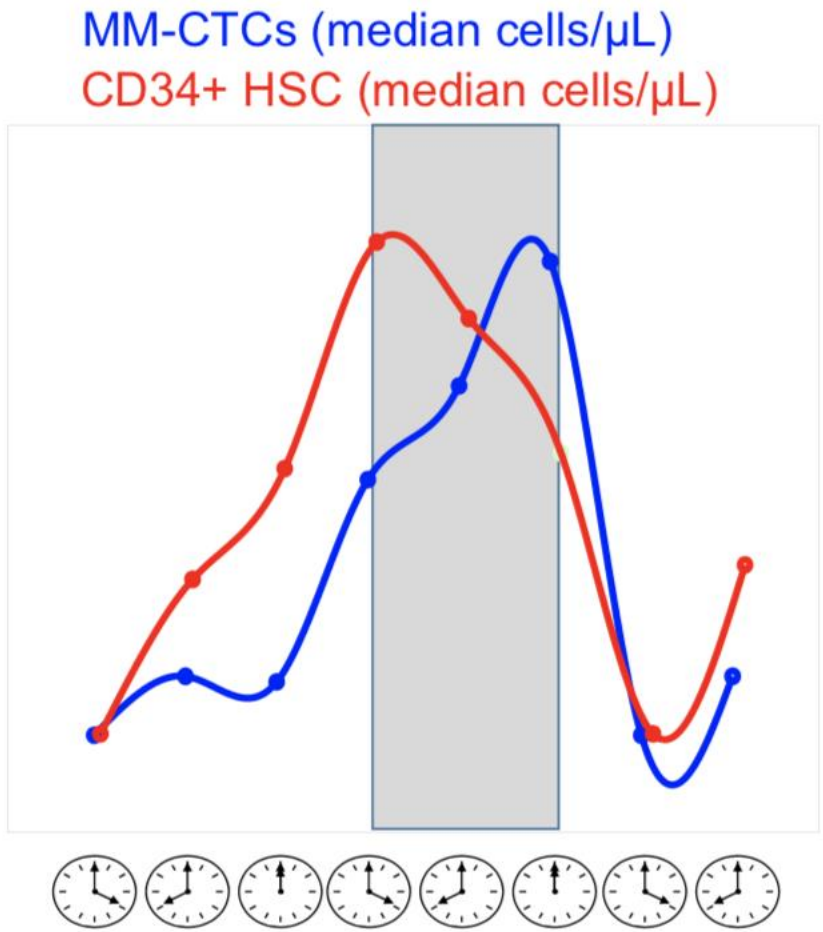


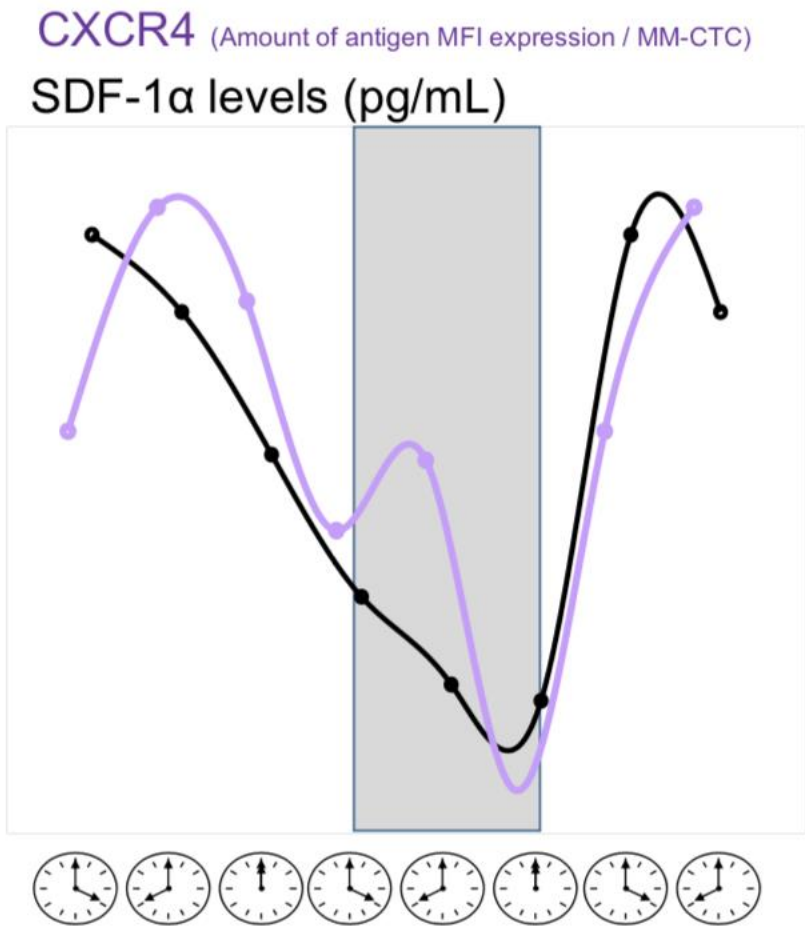
Smoldering MM [2]



1. Kumar. J Clin Oncol. 2005 20;23(24):5668-74
2. Bianchi G, et al. Leukemia. 2013;27(3):680-5

CTCs and CD34+ HSCs share the same circadian rhythm regulated by the SDF1/CXCR4 axis

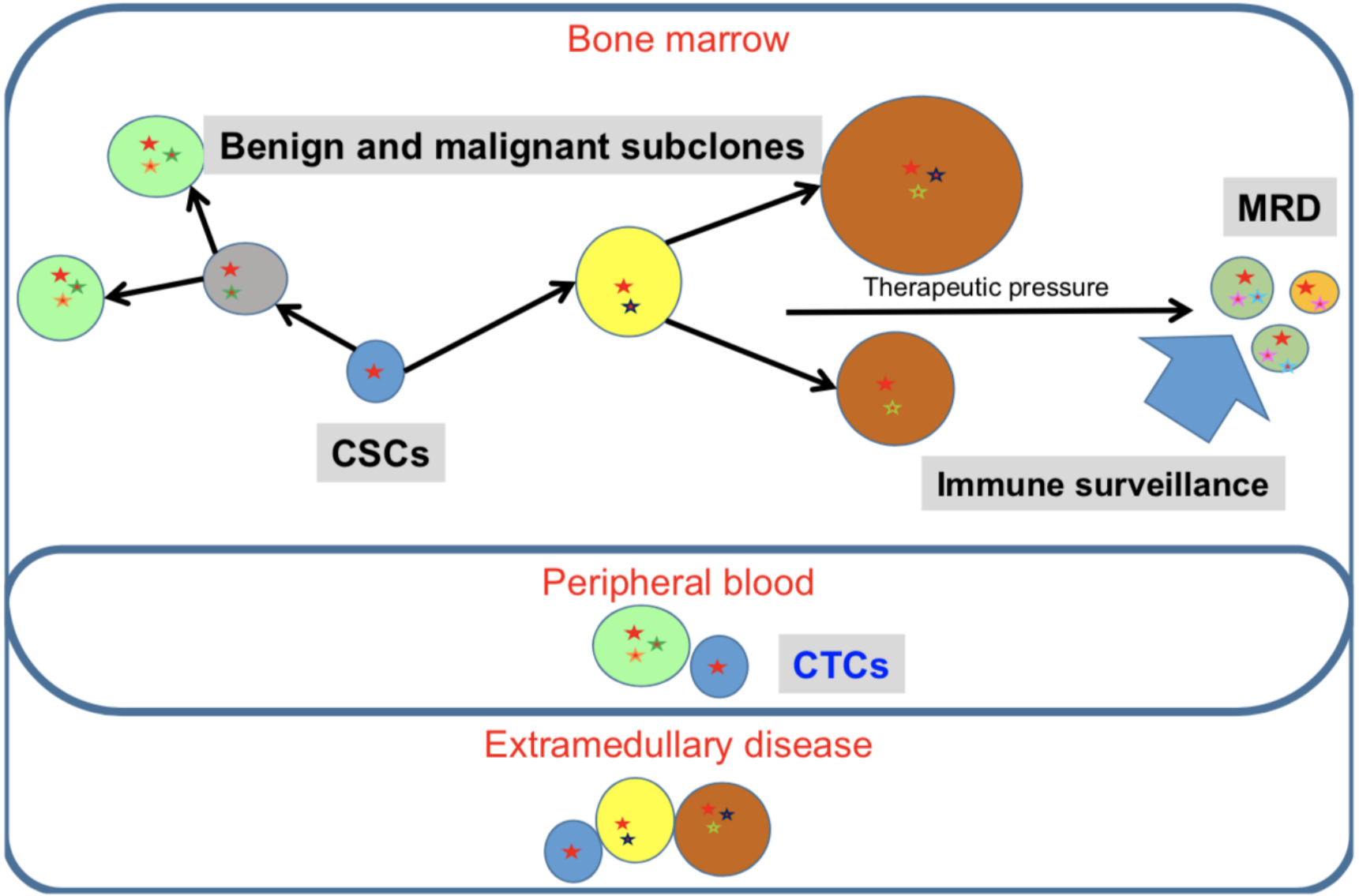




MM patients at relapse (n=6)
Quantification started at 16:00pm every 4h up to 12:00am next day (when patients' initiated treatment)
Time points 16h and 21h have been duplicated to facilitate viewing of the time curve

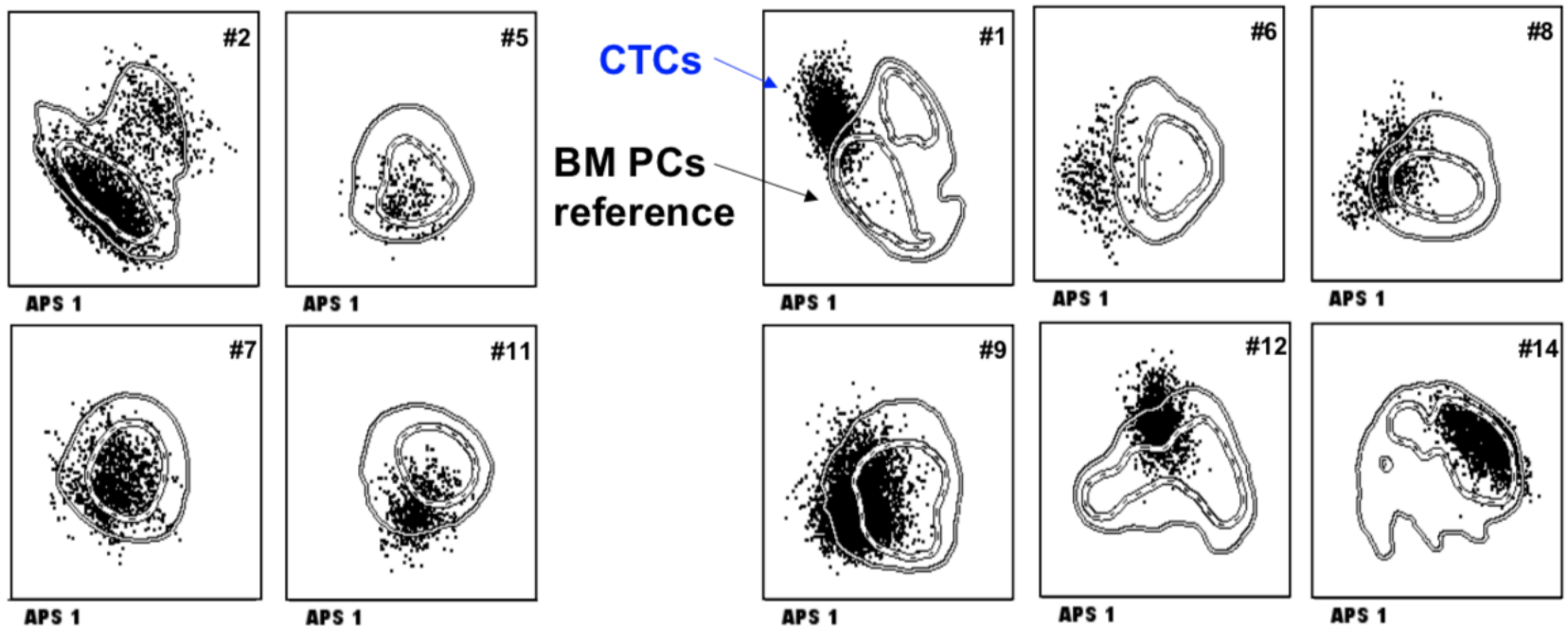
Paiva B, et al. Blood. 2013;122(22):3591-8

Multiple myeloma: a phenotypic perspective



The potential to egress into PB is restricted to a minor subclone in the BM…

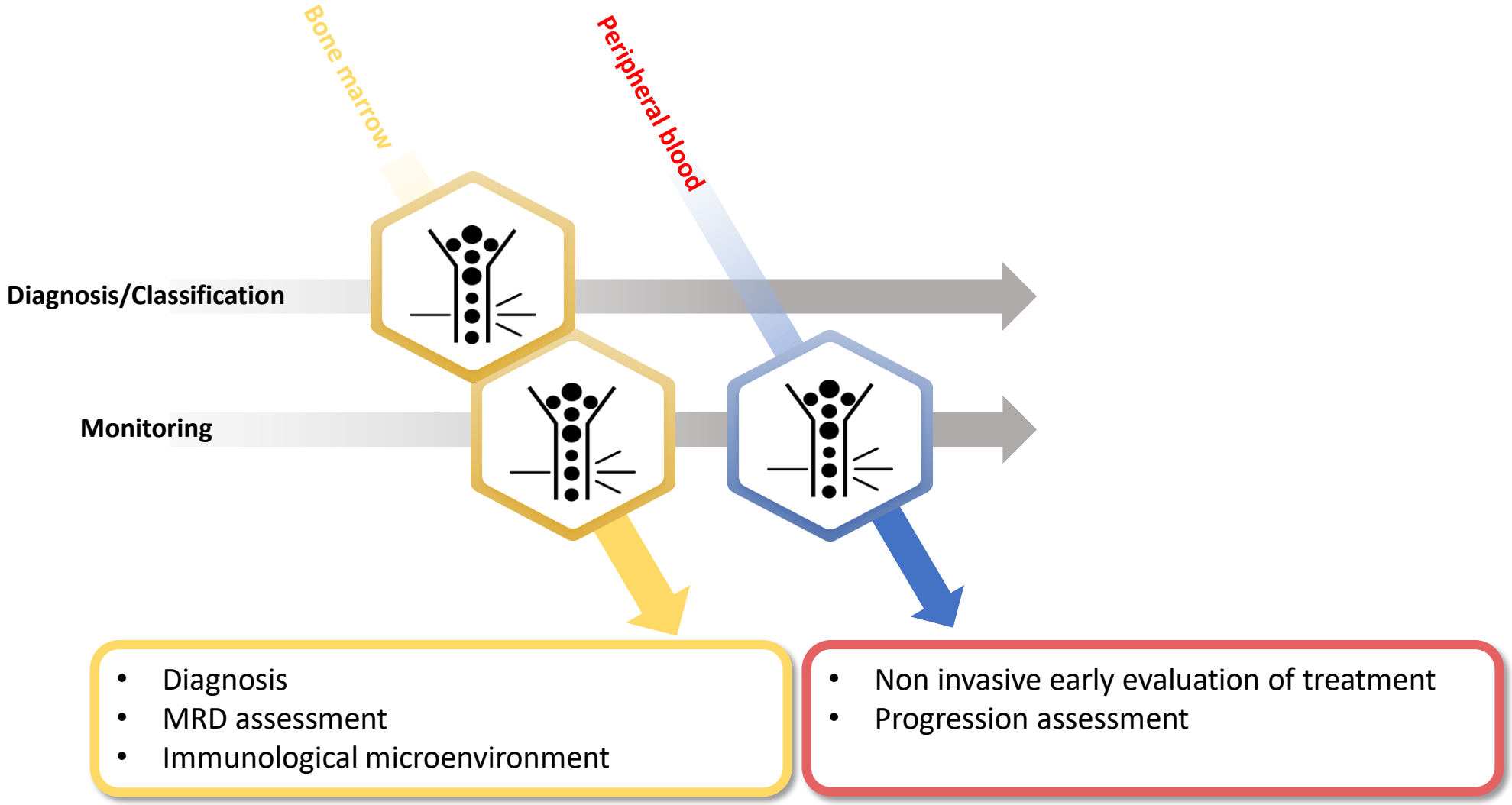
BM MM-PC vs. CTCs: principle component analysis (APS) of 22 antigens



…with an unique profile of integrin and adhesion molecules

Paiva B, et al. Blood. 2013;122(22):3591-8

Diagnosis and Monitoring by Flow Cytometry



Clinical Value

Diagnosis

- Disease staging
- Cell level light chain restriction

Prognosis

- Identification of patients with sustained disease control due to high normal PC recovery and an immune profile with increased B-cell maturation
- Implementation of individualized therapy monitoring strategies

MRD Assessment

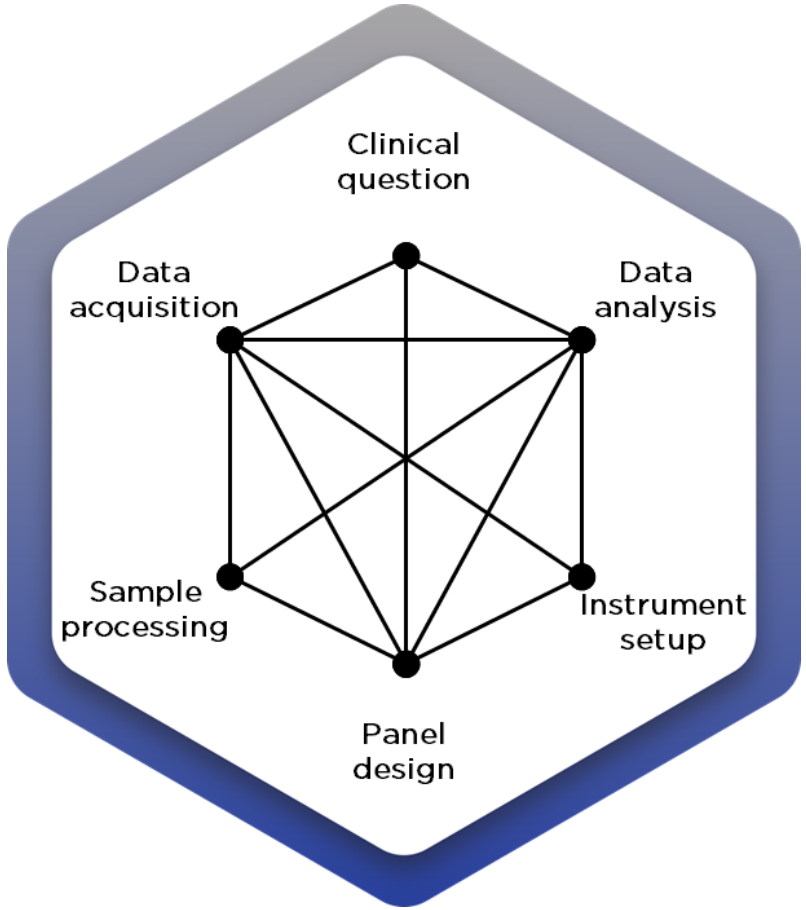
- Detection of abnormal plasma cells at very low levels by highly sensitive technologies
- Compare the efficacy of different treatment strategies

Flores-Montero J, et. al.; Leukemia. 2017 Oct 31(10):2094-2103

NEXT GENERATION FLOW (NGF)

Clinical Question

Panel Design	Meaningful markers Best Conjugates
Instrument Setup	Calibration Compensation
Sample Processing	BulkLysis™ for sample concentration Standard operating procedures (SOP)
Data Acquisition	Required sensitivity - number of events acquired Acquisition speed
Data Analysis	Precise identification of all normal cells Characterization of abnormal phenotypes and distributions



ADVANTAGES NGF

Flow cytometry evaluation of PCDs

Characterization of the **immune cell distribution** and function.

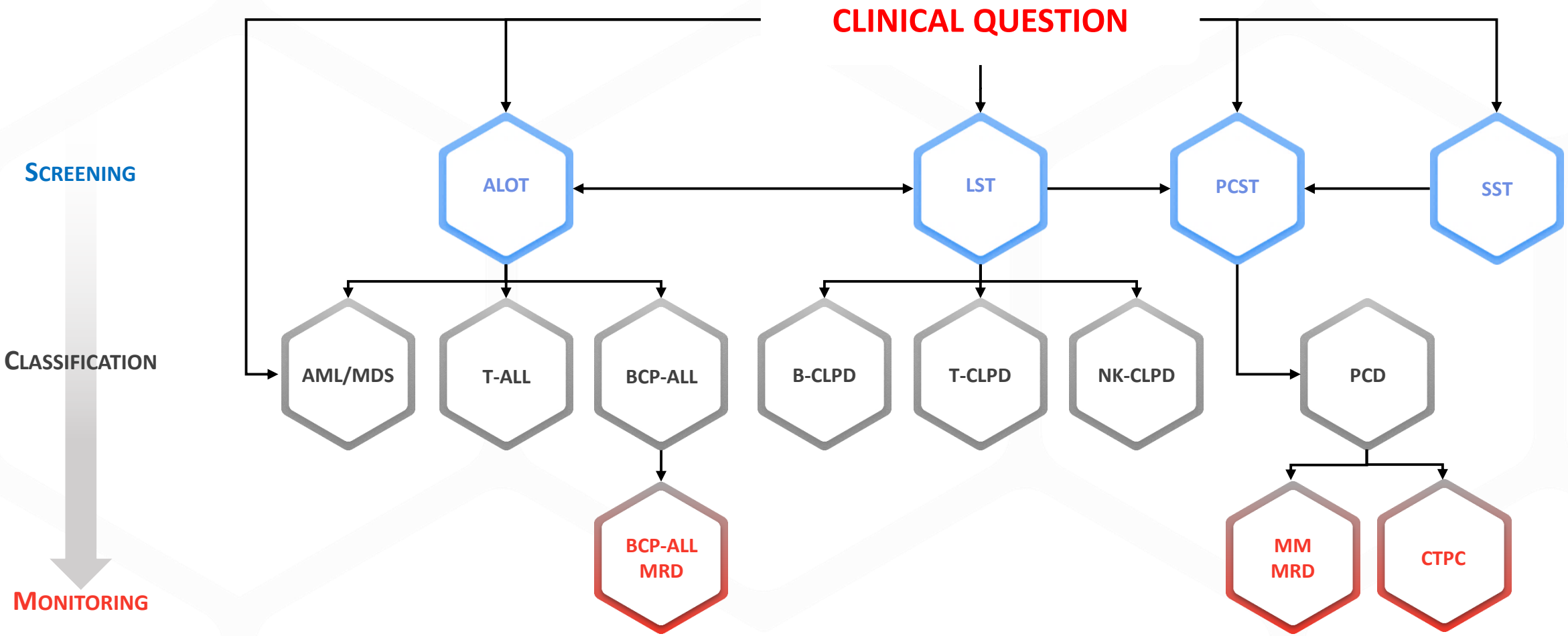
Orientation to specific genetic tests and obtaining of a “probable diagnosis”.

- ✓ Efficient.
- ✓ Widely accessible and minimally invasive.
- ✓ Fast (approximately 4h TAT).
- ✓ Minimizes the need of complete clinical information.

NGF approach for MM MRD evaluation

- ✓ Highly sensitive (20 cells in 10^6) even for those populations with lower frequency.
- ✓ Objective (automated analysis and reporting).
- ✓ Extensively validated by EuroFlow™.
- ✓ External QC assessment available for laboratory performance and sample processing.

EUROFLOW PANELS FOR HEMATO-ONCOLOGY

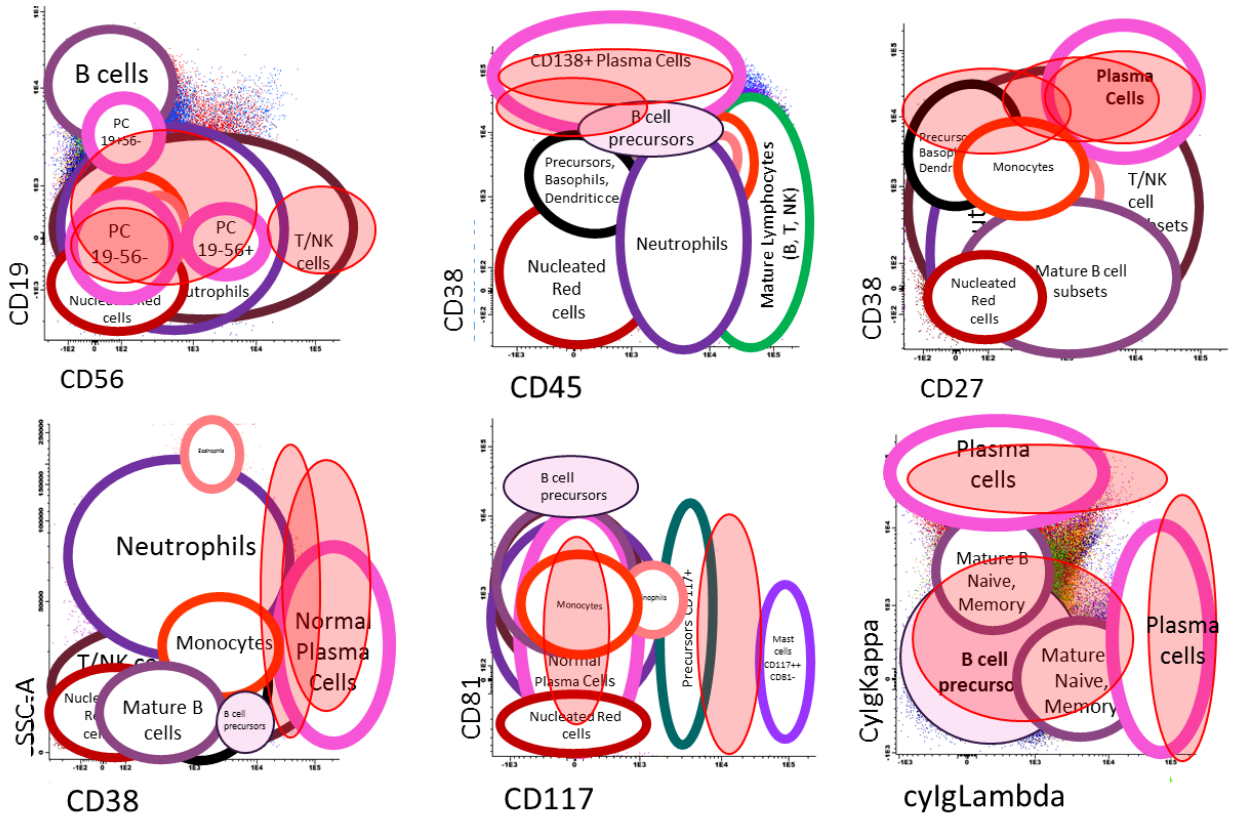


INFINCYT™: ANALYSIS SOFTWARE

MM-MRD Manual Analysis

MANUAL ANALYSIS

- X Subject to Interpretation
- X Multidimensional Complexity
- X Time Consuming

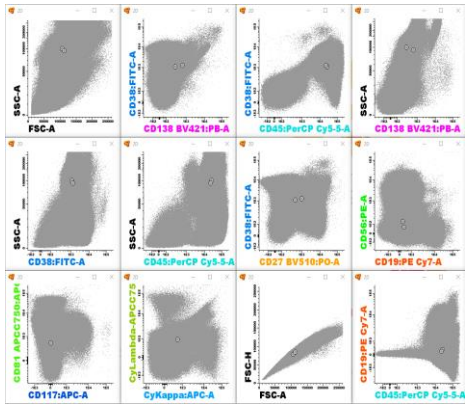


MM MRD automated analysis in Infinicyt

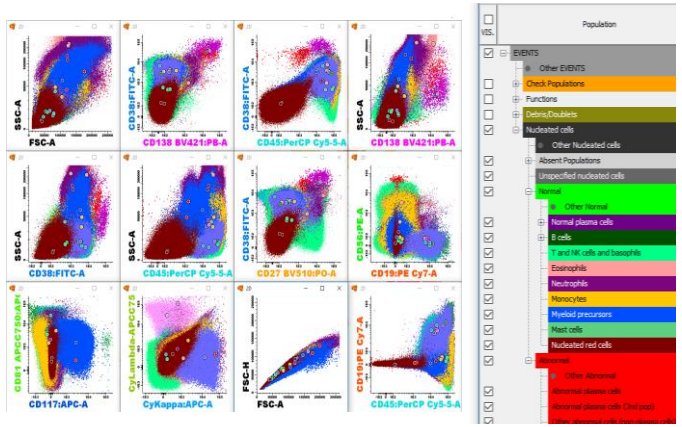
Automated Gating & Identification

Expert user

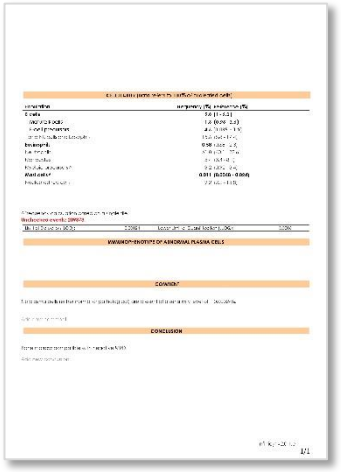
Automatic report



Raw MM MRD flow data



Identified cell populations



Infinicyt identifies normal populations in the sample

The user reviews and confirms what is not normal
(e.g. aberrant plasma cells)

CELLULARITY (Data referred to 100% of the viable cells)

Population	Frequency	Reference	Population	Frequency	Reference
Plasma cells	0.094	(0.048 - 0.97)	Neutrophils	77.3	(60.1 - 78.6)
B cells	1.4	(1 - 5.2)	Monocytes	4.6	(3.4 - 8.1)
Mature B cells	1	(0.95 - 3.6)	Myeloid precursors	1.1	(0.92 - 3.4)
T and NK and basophils	7.7	(8.8 - 17.4)	Mast cells	0.0011	(0.0048 - 0.024)
Eosinophils	1.4	(0.68 - 2.3)	Nucleated red cells	6.8	(2.2 - 15.8)
			Abnormal plasma cells	0.017	-

Resultados estadisticos con referencia de poblaciones normales

Limit of Detection (LOD):	0.00024	Lower Limit of Quantitation (LLOQ):	0.00061

sensibilidad

nuevos conceptos!! **LOD detectable 20** **LOQ cuantificable 50**

ABNORMAL PLASMA CELLS IMMUNOPHENOTYPE

descripción inmunofenotípica de las CPA

$FSC^{normal\ het}$ SSC^{normal} $CD138^{++}$
$CD38^{o\ het}$ $CD56^{+}$ $CD45^{++}$ $CD19^{-}$ $CD27^{lo\ het}$ $CD117^{+}$ $CD81^{+}$
CyIgLambda+

- **marcadores normales de las CPA**
- **marcadores alterados**
- **clonalidad**

COMMENT

comentarios importantes

Plasma cells are present at 0.11% of total nucleated cells: 15.3% of them express an aberrant phenotype ($CD38^{o\ het}$ $CD56^{+}$ $CD45^{++}$ $CD19^{-}$ $CD27^{lo\ het}$ $CD117^{+}$ $CD81^{+}$).
Sample probably hemodiluted (there is a decreased number of mast cells).
Normal plasma cells have altered CyIg K/L ratio. Confirm/discard the normality of these cells.

CONCLUSION

BM compatible with POSITIVE MRD. Note: An hemodiluted sample will underestimate or even fail to detect the percentage of BM infiltration.

AUTOMATED GATING & IDENTIFICATION REPORT

MM MRD automated analysis in Infinicyt

CELLULARITY (Data refers to 100% of viable cells)

Population	Frequency	Reference	Population	Frequency	Reference
Plasma cells (PC)	**0.019**	(0.048 - 0.97)	**Neutrophils**	**58.6**	(60.1 - 78.6)
B cells	2.7	(1 - 5.2)	**Monocytes**	**8.4**	(3.4 - 8.1)
Mature B cells	1.2	(0.95 - 3.6)	Myeloid precursors ^	1.4	(0.92 - 3.4)
B-cell precursors	1.5	(0.085 - 1.9)	**Mast cells ^**	**0.004**	(0.0048 - 0.024)
T and NK and basophils	**20.6**	(8.8 - 17.4)	Nucleated red cells	7	(2.2 - 15.8)
Eosinophils	2	(0.68 - 2.3)	**Abnormal PC**	**0.0073**	-

^Frequency calculation based on a single file.

Limit of Detection (LOD):	0.00022	Lower Limit of Quantification (LLOQ):	0.00056

IMMUNOPHENOTYPE OF ABNORMAL PLASMA CELLS

$CD81^{-}CD138^{++}$
$CD117^{+}CD27^{-}CD38^{lo}FSC^{hi\,het}CD19^{-}CD56^{+}CD45^{-}SSC^{hi}$
CylgKappa+

COMMENT

0.026% of Plasma cells were detected: 27.9% of these express an aberrant phenotype ($CD117^{+}CD27^{-}CD38^{lo}FSC^{hi\,het}CD19^{-}CD56^{+}CD45^{-}SSC^{hi}$).
Sample possibly contaminated with peripheral blood (decreased percentage of mast cells was observed).

CONCLUSION

Bone marrow compatible with positive MRD. Note: Possible hemodilution of the sample must be considered to assess the percentage of infiltration.

MM-MRD Assessment

- Abnormal Plasma Cells

Quality of the Sample: Hemodilution

- Mast Cells

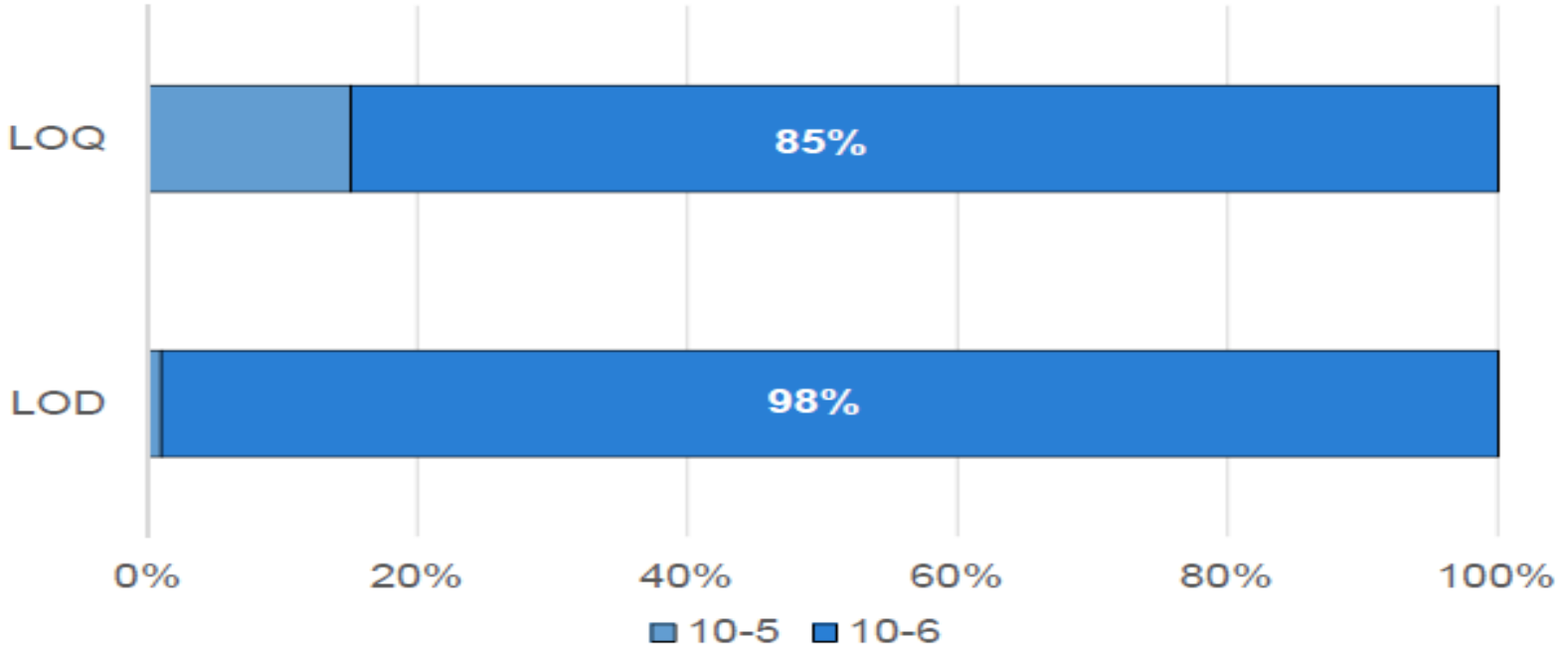
Sensitivity

- Number of events

Bone Marrow Immune Regeneration

- B-Cell Precursors
- Nucleated Red Cells

NGF reaches 10^{-6} sensitivity in the vast majority of MM patients (GEM2012MENOS65)

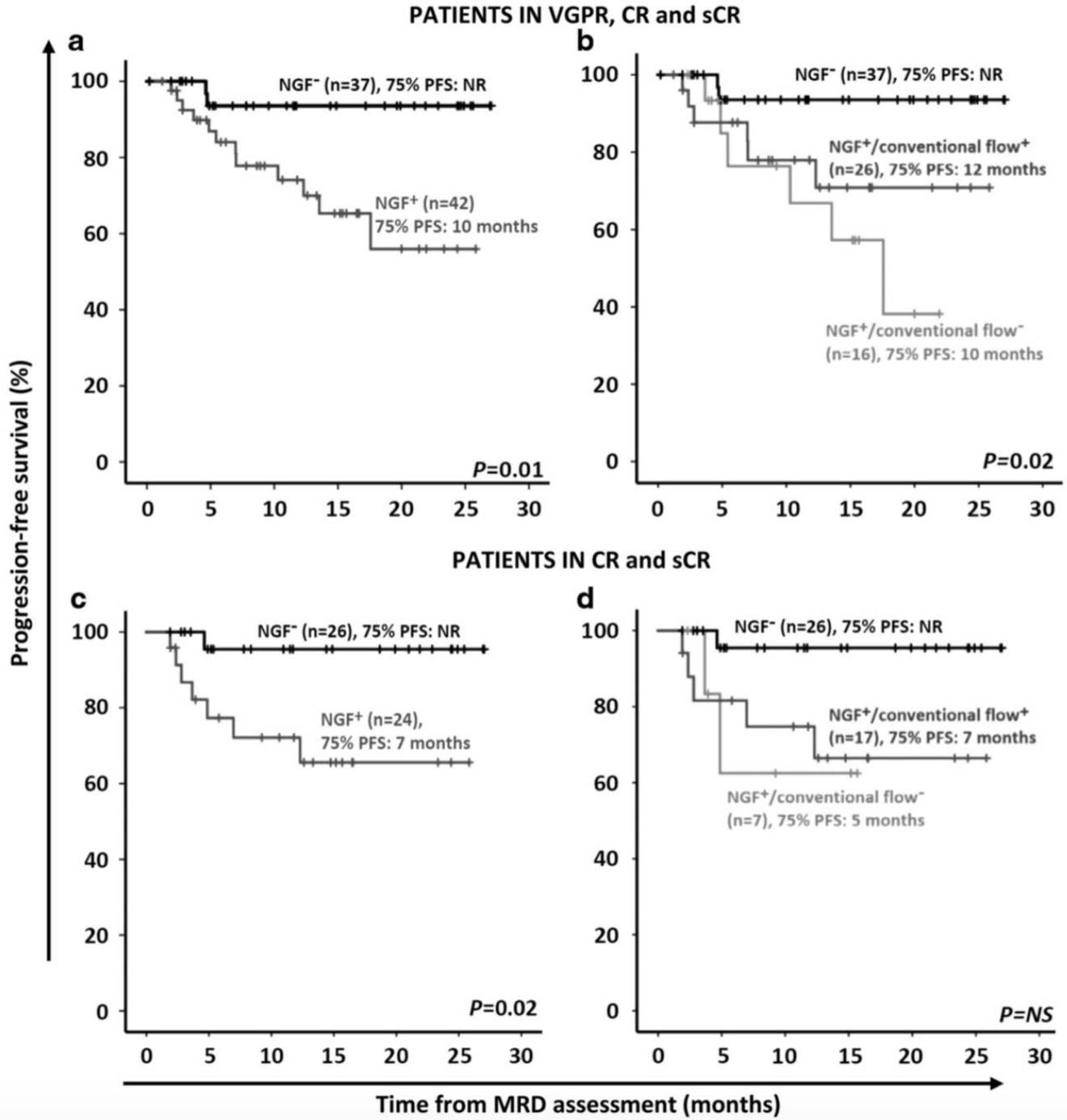


LOQ (level of quantitation) : 50 cells / total nucleated viable cells
LOD (level of detection): 20 cells / total nucleated viable cells

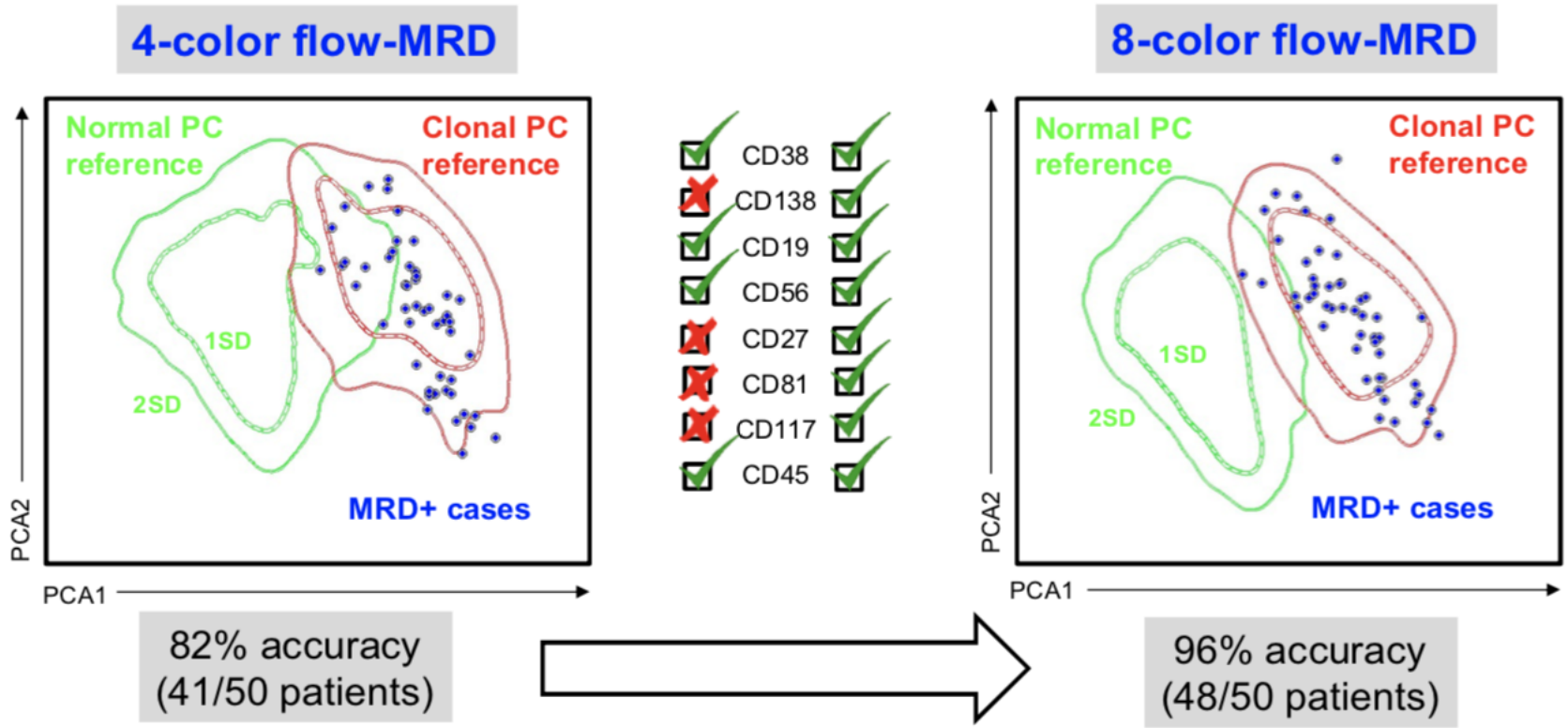
Flores-Montero J, et al. Leukemia. 2017. doi: 10.1038/leu.2017.29

LOQ: límite de cuantificación: 50 células/ total de células nucleadas viables

LOD: límite de detección: 20 células/ total de células nucleadas viables


PATIENTS IN VGPR, CR and sCR
a
NGF⁻ (n=37), 75% PFS: NR
NGF⁺ (n=42)
75% PFS: 10 months
P=0.01
b
NGF⁻ (n=37), 75% PFS: NR
NGF⁺/conventional flow⁺
(n=26), 75% PFS: 12 months
NGF⁺/conventional flow⁻
(n=16), 75% PFS: 10 months
P=0.02
PATIENTS IN CR and sCR
c
NGF⁻ (n=26), 75% PFS: NR
NGF⁺ (n=24),
75% PFS: 7 months
P=0.02
d
NGF⁻ (n=26), 75% PFS: NR
NGF⁺/conventional flow⁺
(n=17), 75% PFS: 7 months
NGF⁺/conventional flow⁻
(n=7), 75% PFS: 5 months
P=NS
Progression-free survival (%)
Time from MRD assessment (months)

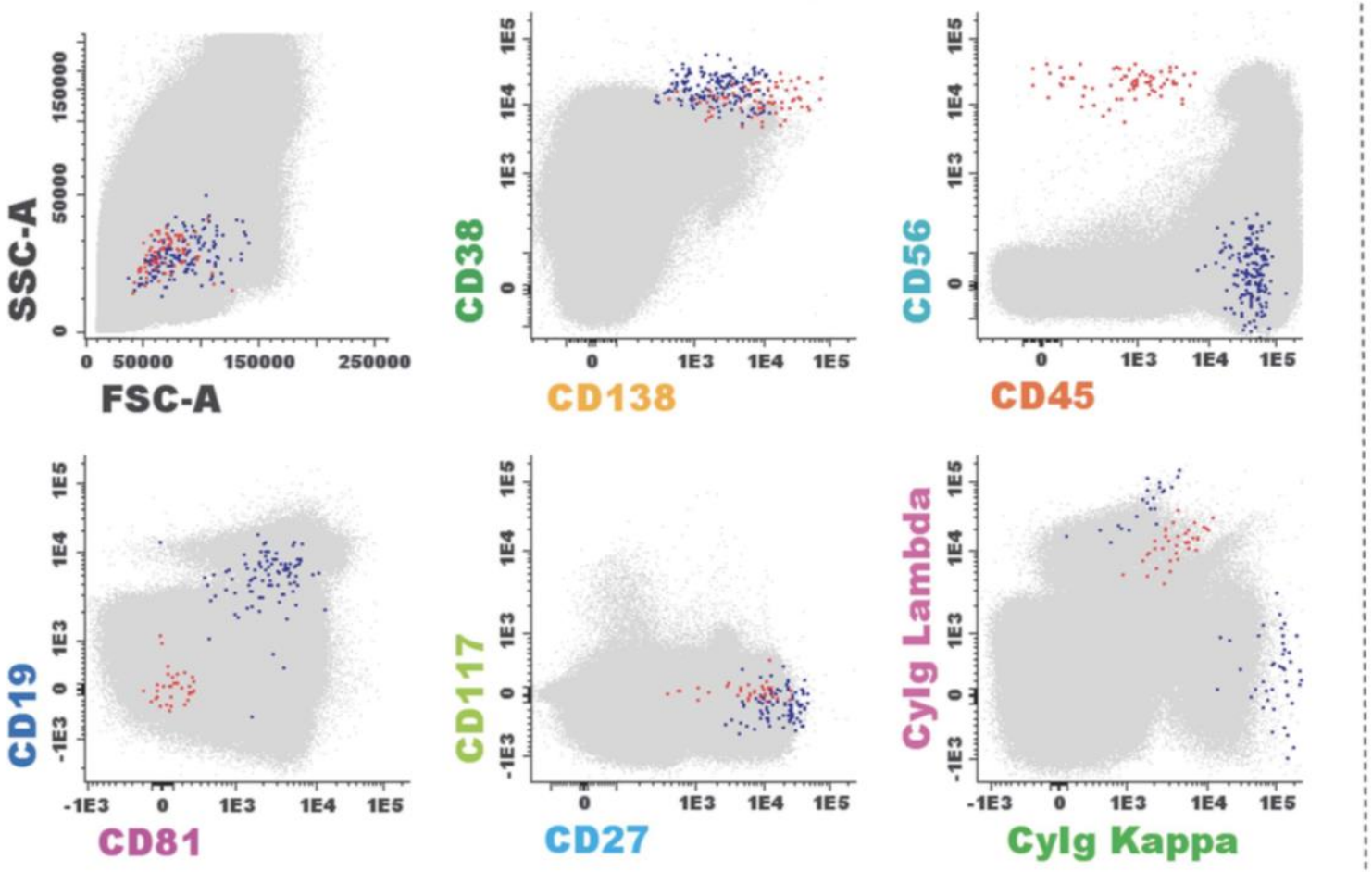
2016: MRD monitoring using 2nd generation flow improves discrimination between normal vs. clonal PCs



GEM2010MAS65

Paiva B et al. Blood 2016;127(25):3165-74

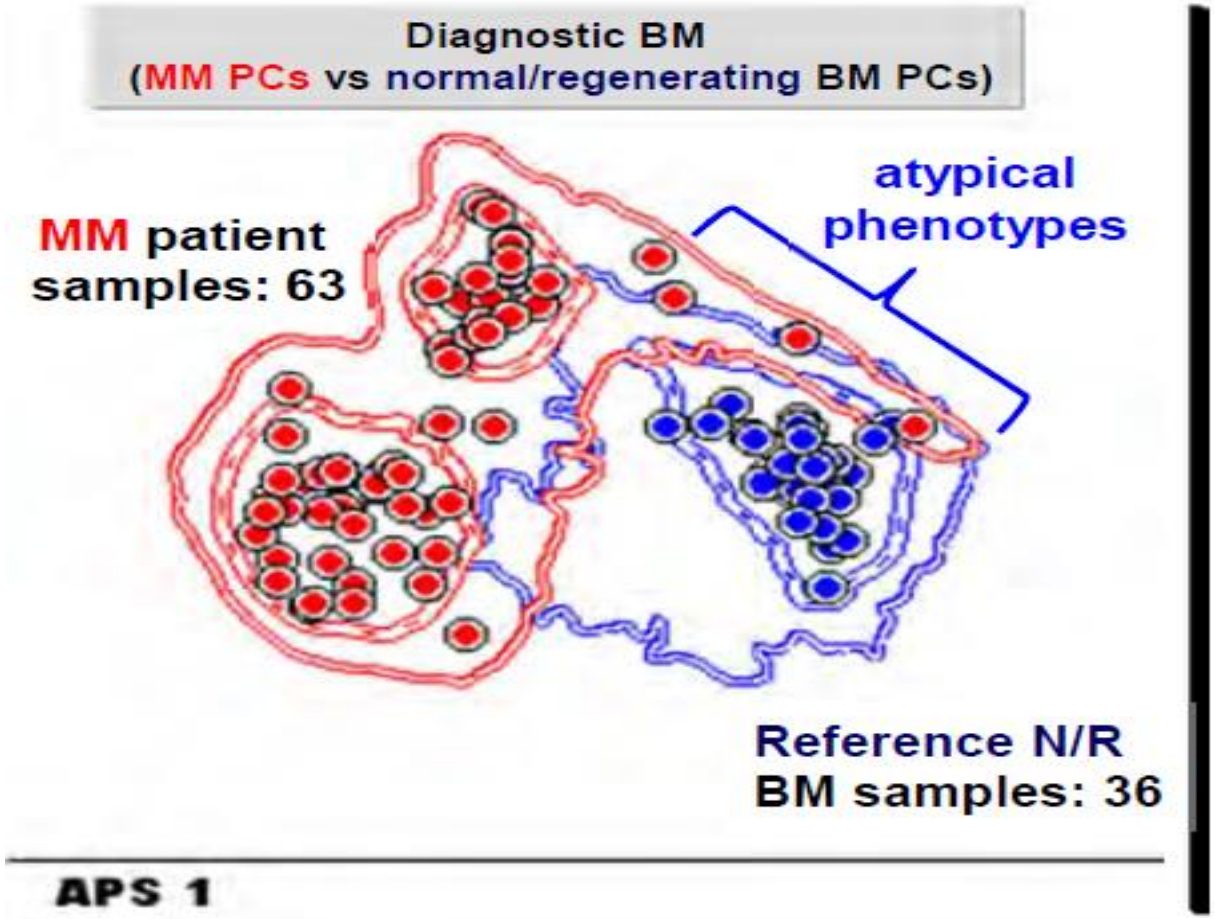
Next gen flow-minimal residual disease in myeloma
J Flores-Montero *et al*

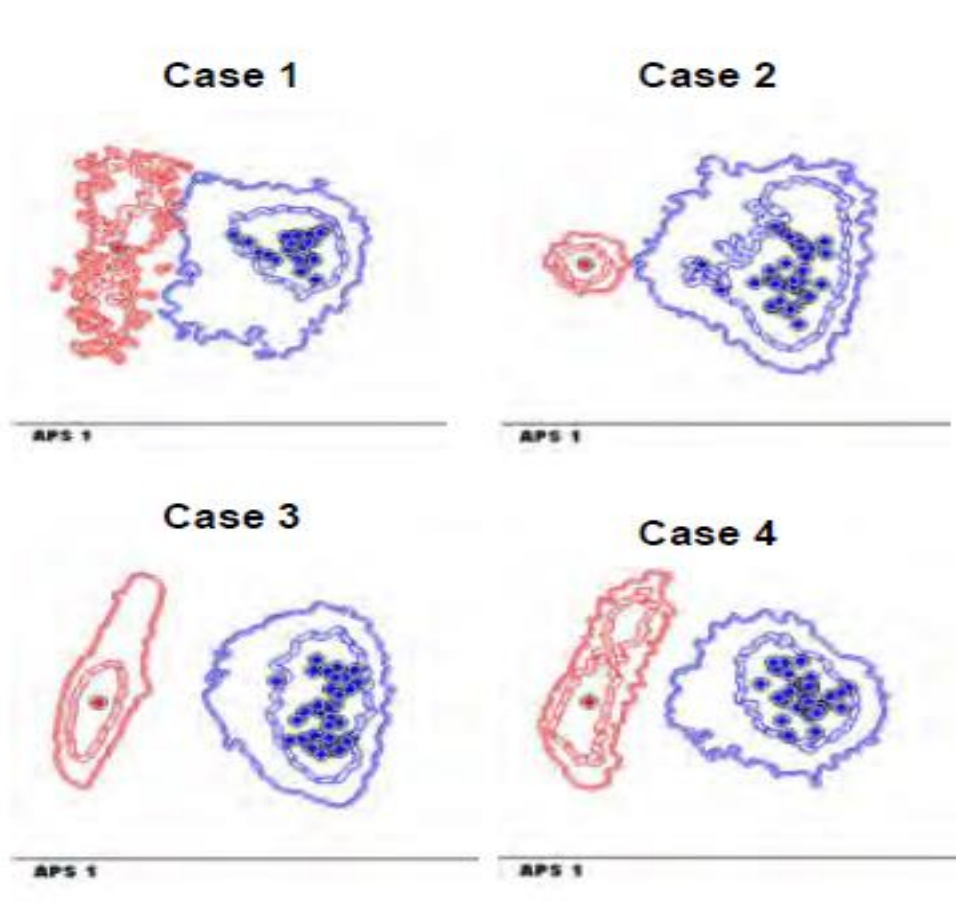


Parameter	% of contribution
CD56	28%
CD19	19%
CD45	17%
CD81	16%
CD138	6%
CD27	4%

- **Muestra óptima: médula ósea con EDTA, sin hemodilución**
- **Cantidad de células disponibles:adquirir 10 millones!!!**

Next generation Flow-MRD monitoring in MM
- applicable to all patients -





New software: Principal component analysis: clusters

Flores-Montero J, et al. Leukemia. 2017. doi: 10.1038/leu.2017.29

Conclusions

-**MRD positive or negative:** Compatible with MM MRD positive conclusion will be reported for values above the LOD.

-**Warning about the quality of the sample (hemodilution):** The criteria used for hemodilution is the frequency of mast cells lower than the range of the database.

Note: Mesenchymal cells were also considered, but finally were discarded because this panel could not accurately identify them. Nucleated red cells were also considered but finally discarded because there is a great heterogeneity within normal samples. Mast cells population is more stable and homogeneus, it can be affected by treatment but it is not very common.

-**Warning about the number of events acquired**: Recommended standard about number of events to be acquired is 10 Million to reach a sensitivity of 10^{-5}. It takes into account an acquisition of 10 Millions with typically 20% of debris. The warning appears if less than 8 Million of nucleated cells if found at the end of analysis.

Gracias por su atención!!!